The Grazing Impact of Microzooplankton on Phytoplankton of Different Size Classes in Tolo Harbour and Mirs Bay, Hong Kong

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Abstract

The aim of this study was to compare the microzooplankton grazing rates between Tolo Harbour (TH), a eutrophic bay with high nutrient concentrations and frequent algal bloom, and Mirs Bay (MB), where nutrient concentrations were much lower. The goal was to provide preliminary insights into the microbial food web dynamics in these two sites.

Microzooplankters are heterotrophic planktonic organisms in the 20 – 200 μm size range. They are major grazers of phytoplankton because of their high abundance, ubiquitous distributions, fast growth rates, and also their ability to ingest a large size range of food particles. But despite their feeding plasticity, they have been found to prefer smaller phytoplankton. Higher proportions of small phytoplankton are generally considered to be an important feature of areas with lower nutrients and chlorophyll concentrations. A hypothesis that Mirs Bay has a high proportion of small phytoplankton and therefore higher microzooplankton grazing rates was hence established.

The dilution method was used to estimate the phytoplankton growth rates and microzooplankton growth rates in TH and MB. High performance liquid chromatography and phytoplankton size fractionation (< 200 μm, < 20 μm and < 5 μm) were incorporated with the dilution method to provide additional information on microzooplankton feeding preference towards phytoplankton of different taxa and size classes.

Despite large differences in average chlorophyll *a* concentrations between the two sites (10.27 μg L^{-1} in TH and 0.82 μg L^{-1} in MB), proportions of < 5 μm phytoplankton were on average surprisingly similar (50.3% in TH and 59.9% in MB). The hypothesis could not be tested because of these results. Phytoplankton growth rates (-0.17 – 2.44 d^{-1} in TH and 0.11 – 2.87 d^{-1} in MB) and microzooplankton

grazing rates (0.58 – 2.26 d^{-1} in TH and 0.61 –1.49 d^{-1} in MB) were also comparable between the two sites. Microzooplankton grazing impact was higher in TH mainly because microzooplankton grazing rates were usually higher than phytoplankton growth rates. Our results do not support any microzooplankton selectivity towards phytoplankton size, but heavy grazing on alloxanthin despite its low growth rate suggested possible preference towards cryptophytes.

The dilution method proved to be a convenient tool for studying micrograzers feeding dynamics, but the exclusion of mesograzers may have lead to inaccurate estimations of the *in-situ* phytoplankton grazing mortality.

摘要

此研究的目的是要比較微型浮游動物對浮游植物在香港吐露港和大鵬灣的攝食. 吐露港位於香港東北部, 其水質有高養份及經常產生紅潮的特徵. 大鵬灣連接著吐露港, 但其水質相對上比吐露港較少養份. 這些研究結果能爲這兩個研究場所的微生物食物網的互動提供初步的見解.

微型浮游動物是身型位於 20 – 200 μm 之間的異養浮游動物. 牠們是浮游植物的主要攝食者, 因爲牠們數量龐大, 分佈廣闊, 增長率快速, 及有攝取不同尺寸食物的能力. 但是,儘管他們的攝食範圍有極大的可塑性, 牠們常被發現偏向選取較小的浮游植物. 而較小的浮游植物通常會在低養分和葉綠素濃度的水域有較高的比例. 此研究假設大鵬灣應該有高比例的較小浮游植物, 因此有較高的微型浮游動物攝食影響.

研究方法運用了能同時爲吐露港(TH)和大鵬灣(MB)兩個研究地點測量浮游植物生長率和微型浮游動物攝食率的稀釋法. 同時研究方法亦融合高效液相色譜法和粒級分離(< 200 μm, < 20 μm and < 5 μm)於稀釋法中, 藉此了解微型浮游動物對浮游植物大小及種類的攝食偏向.

雖然兩個研究地點平均葉綠素 *a* 濃度差距大(TH: 10.27 μg L^{-1}; MB: 0.82 μg L^{-1}), 兩地< 5 μm 葉綠素 *a* 的比例出奇地相似(TH: 50.3%; MB: 59.9%). 因爲這些結果, 我們的假設不能確實地被驗證. 兩地的浮游植物生長率(TH: -0.17 – 2.44 d^{-1}; MB: 0.11 – 2.87 d^{-1})和微型浮游動物攝食率(TH: 0.58 – 2.26 d^{-1}; MB: 0.61 –1.49 d^{-1})亦相似. 微型浮游動物攝食影響可從微型浮游動物攝食率對浮游植物生長率的比率來推測. TH 的微型浮游動物攝食影響較 MB 高, 因那裡的微型浮游動物攝食率通常比其浮游植物生長率高. 我們的實驗結果不能證實微型浮游動物對較小浮游植物有偏食, 但微型浮游動物對低生長率的異黃素有較高的攝食率, 這暗示了本地的微型浮游動物可能對穩芽植物有偏好.

稀釋法雖然被證明能方便地提供有關微型浮游動物攝食的數據, 但因此方法排除了中型浮游動物, 可能會導致對浮游植物於實際生態中的死亡率測量不

準確.

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Chapter 1

Introduction

1.1. Microzooplankton

Microzooplankton is defined as heterotrophic or mixotrophic plankton in the 20 – 200 μm size range (Sieburth et al. 1978, Calbet 2008). This definition includes a diverse group of organisms with various sizes, taxonomic groups, and trophic relationships (Landry & Calbet 2004). Microzooplankters, especially protozoans, are characterized by their high abundance (often > 1000 L^{-1}) (Burkill et al. 1995), ubiquitous distribution (Capriulo 1991), fast growth rates (e.g. Jonsson 1986, Bernard & Rassoulzadegan 1990), and ability to ingest a large size range of food particles (e.g. Hansen et al. 1994, Peters 1994).

The feeding plasticity of microzooplankters owe in large to their various, and sometimes unique feeding mechanisms. Besides phagocytosis, the feeding mechanism commonly employed by protozoans, heterotrophic dinoflagellates can also perform tube feeding in which a feeding appendage is used to suck the contents of their prey and pallium feeding in which thecate heterotrophic dinoflagellates use a plastic feeding membrane called pallium, to envelop and digest food particles extracellularly (reviewed by Hansen and Calado 1999)

1.1.2. Microzooplankton grazing

Due to the high abundance, growth rate and feeding plasticity of microzooplankton (See section 1.1), they have long been considered as important grazers on phytoplankton (Pomeroy 1974). But due to their size overlap with phytoplankton, direct microzooplankton grazing experiments were hard to conduct in the past (Landry & Hassett 1982, Gifford 1988). As seen in table 1.1, before the

Table 1.1. Summary of methods used to estimate the grazing impact of microzooplankton (modified from Gifford 1988).

Method	Advantage(s)	Disadvantage(s)	Reference(s)
Indirect methods			
1 Correlation of natural consumer-prey cycles	- Non-invasive	- Qualitative	Smetaček 1981, Sheldon et al. 1986
2 Extrapolation of laboratory rates to the field	- Non-invasive	- May not represent *in situ* conditions	Beers & Stewart 1970, 1971, Taguchi 1976, Heinbokel 1978, Rassoulzadegan & Etienne 1981, Burkill 1982, Rassoulzadegan 1982, Capriulo & Carpenter 1983, Hernroth 1983, Cosper & Stepien 1984, Andersen & Sorensen 1986, Paranjape et al. 1985
3 Extrapolation from other field data	- Non-invasive	- Correlations may not reflect natural relationships	Riley 1956, Takahashi & Hoskins 1978
Direct methods			
1 Tracers of ingestion			
(A) Inert particles	- Quantitative - Demonstrates phagocytosis directly	- Selective feeding by consumers may affect results	Heinbokel & Beers 1979, Børsheim 1984

Table 1.1. (Continued)

Method	Advantages	Disadvantages	Reference(s)
Direct methods			
1 Tracers of ingestion			
(B) Radioisotopes	- Quantitative - Sensitive	- Alternate pathways of isotope uptake affect cycling of tracer - Highly manipulative	Lessard & Swift 1985
(C) Fluorescently labeled bacteria	- Quantitative	- Limited to bacterivory only	Sherr et al. 1987
2 Metabolic inhibitors	- Quantitative	- Non-specificity of inhibitors	Campbell & Carpenter 1986
3 Size fractionation	- Quantitative - Uses natural assemblage	- No true controls - Highly manipulative - Predators & prey are not unequivocally separated	Capriulo & Carpenter 1980, Verity 1986
4 Seawater dilution	- Quantitative - Simultaneous estimation of algal growth & mortality - Minimally manipulative to natural assemblage	- May alter natural assemblage - Unproven assumption that feeding thresholds do not occur	Landry & Hassett 1982, Burkill et al. 1987, Paranjape 1987
5 Pigment budget	- *In situ* - No manipulation of natural assemblage	- Uncertainty of conversion efficiency of chlorophyll to phaeopigments	SooHoo & Kiefer 1982, Welschmeyer & Lorenzen 1985

1980s, most microzooplankton grazing estimates were indirect and might not reflect natural conditions. Various methods for direct estimation of microzooplankton grazing emerged during the early 1980s, and all having different advantages and disadvantages. Despite the number of new methods available for microzooplankton grazing estimation, the dilution method or experiment, also known as seawater dilution or serial dilution, introduced by Landry and Hassett in 1982, stood out amongst the other methods and has essentially become the standardized method for estimating microzooplankton grazing due to its simplicity and various advantages (Dolan et al. 2000, Calbet 2008).

1.2. Dilution method

1.2.1. Basic principles

The dilution method involves the use of particle free water to dilute "whole" water from the same source. The principle of the method is to manipulate the density of primary consumers through dilution, reducing encounter rates between prey and grazers, eventually affecting the grazing pressure. The apparent growth rates of phytoplankton in different dilutions together with the dilution factor can then be used to calculate both the true growth rate and the mortality rate of phytoplankton, which is assumed to be mostly due to grazing.

The method relies on three important assumptions:

1. Phytoplankton growth is exponential.
2. Phytoplankton growth is independent of phytoplankton density.
3. Microzooplankton grazing rate is solely dependent on encounter rate with phytoplankton, and thus changes linearly with the dilution factor.

Assumption 2 may be violated easily when nutrients required for phytoplankton growth becomes limiting in incubations with low dilution factor and high

phytoplankton density. Supplementary nutrients are therefore often supplied to all incubations to ensure that phytoplankton growth is not limited by nutrient depletion. As examples, table 1.2 presents the levels of nutrient addition in dilution experiments conducted by several investigators.

By assuming that phytoplankton growth is exponential, the change in phytoplankton density (P) over a time period (t) can be expressed as:

$$P_t = P_o\, e^{(\mu-g)t} \qquad (1)$$

Where μ and g are the phytoplankton growth and grazing mortality rate respectively. P can be expressed in different units such as cell densities (e.g. Kuipers & Witte 1999), or most commonly chlorophyll *a* concentrations (e.g. Landry & Hassett 1982, Safi et al. 2007). Theoretically, it is also possible to use phytoplankton biomass as P, but since chlorophyll *a* concentration or cell densities are needed to calculate biomass, few investigators go through the trouble of converting these two measurements into biomass just to obtain phytoplankton growth and grazing mortality rates. By rearranging equation (1), phytoplankton apparent growth rate can be expressed as:

$$\text{Apparent phytoplankton growth rate} = 1/t \ln (P_t / P_0) = \mu - g \qquad (2)$$

According to assumption 3, g is modified by the dilution factor (D), so that equation (2) becomes:

$$\text{Apparent phytoplankton growth rate} = 1/t \ln (P_t / P_0) = \mu - Dg \qquad (3)$$

Therefore, by regressing apparent phytoplankton growth rate against D, μ and g can be determined as the y-intercept and slope of the curve. (Landry & Hassett 1982, Landry 1993). By using the estimated μ and g together with the initial phytoplankton biomass (B_0), it is also possible to estimate the phytoplankton primary production (PP) with the dilution experiment (Moigis & Gocke 2003):

$$PP = \mu(\mu - g)^{-1} ([B_0 e^{(\mu-g)t}] - 1)$$

Table 1.2. Summary of the amounts of nutrients added in past dilution experiments.

Region	Nutrients added (μM)	Reference
Coastal Washington	N: 10 P: 1	Landry & Hassett 1982
Celtic Sea	N: 5 P: 1	Burkill et al. 1987
Central equatorial Pacific	NH_4^+: 0.5 P: 0.03 $FeSO_4$: 0.0001	Landry et al. 1995a
Northern Gulf of Mexico	NH_4Cl: 5 – 10 KH_2PH_4: 0.5 – 1	Strom & Strom 1996
Arabian Sea	NH_4^+: 0.5 PO_4^{2-}: 0.03 $FeSO_4$: 0.0001 $MnSO_4$: 0.0001	Landry et al. 1998
North Atlantic	N: 10 P: 1 $Si(OH)_4$: 5 $MnCl_2$: 0.01 $FeSO_4$: 0.1	Gaul & Antia 2001
Korea	N: 20 P: 2 $MnSO_4$: 0.0001	Kim et al. 2007
Coastal Texas	NH_4Cl: 4 – 8 Na_2HPO_4: 0.3 – 0.6	First et al. 2007
South Brazil	N: 9	McManus et al. 2007
Coastal Northwestern Mediterranean	NH_4Cl: 15 Na_2HPO_4: 1	Calbet et al. 2008

Calbet and Landry (2004) tested results of *PP* calculated from dilution experiments with those obtained from ^{14}C, the standard test for estimating *PP*, and had found that the two values are well related, demonstrating the reliability of the results obtained from dilution experiments.

Microzooplankton grazing impact (*G*) expressed as the proportion of *PP* consumed can be shown through the ratio *G*:*PP* where *PP* and *G* are in turn calculated using the equations (Landry et al. 2000):

$$PP = \mu P_m \quad \text{and} \quad G = gP_m$$

Where P_m is the mean phytoplankton concentration during incubation. Since

$$\frac{G}{PP} = \frac{gP_m}{\mu P_m}$$

Therefore, $G{:}PP = g{:}\mu$.

Due to the design of the dilution experiment, it does not discriminate between different types or sizes of grazers (Calbet 2008). The microzooplankton grazing rate estimated is in terms of the community instead of individual grazers (Strom & Welschmeyer 1991), and although the grazers are conventionally referred to as microzooplankton, which by denfinition are between the size range 20 – 200 μm (See section 1.1), the grazers in dilution experiments includes all grazers < 200 μm.

1.2.2. Variation and extensive uses of the dilution method

The use of the dilution method has been modified and extended to the study of various aspects of microzooplankton grazing. This is usually done by replacing other parameters instead of chlorophyll *a* as phytoplankton density (*P*) (See section 1.2).

The incorporation of high performance liquid chromatography (HPLC) with the dilution method was first introduced by Burkill et al. in 1987 to study microzooplankton grazing on various taxonomic groups of phytoplankton. HPLC

enables the rapid detection of large numbers of phytoplankton pigments in addition to chlorophyll *a*, some of which can be used as chemotaxonomic markers for certain phytoplankton taxonomic groups (See section 1.4.2 and tables 1.4 – 1.6). This provides information of microzooplankton grazing on not only the general phytoplankton community, but individual taxa that can be identified by pigment markers as well. Since 1987, many have used HPLC to obtain pigment-specific grazing rates (e.g. Strom & Welschmeyer 1991, McManus and Ederingtoncantrell 1992, Verity et al. 1993, Waterhous & Welschmeyer 1995, Latasa et al. 1997, Gaul & Antia 2001, Suzuki et al. 2002, Fileman et al. 2002, Obayashi & Tanoue 2002, Strom and Welschmeyer 1991, Waterhouse and Welschmeyer 1995, Gaul and Antia 2001, Fileman et al. 2002, Obayashi and Tanoue 2002, Suzuki et al. 2002, Landry et al. 2003, Palomares-García et al. 2006). The phytoplankton community can also be divided into different size fractions (See section 1.4.1), and there are various studies that measure the composition of different size fractions in the samples (e.g. Caron et al. 2000, Suzuki et al. 2002, Kim et al. 2007), as well as actual grazing rates data on different phytoplankton size fractions (e.g. Froneman & McQuaid 1997, Kuipers and Witte 1999, Strom et al. 2001, Zhang et al. 2005, Safi et al. 2007).

Besides phytoplankton, the dilution method has also been used to study the microzooplankton grazing impact on the bacterioplankton community (e.g. Tremaine & Mills 1987, Rivkin et al. 1999, Anderson & Rivkin 2001) by replacing *P* with bacterioplankon density. This application, however, is not as popular due to various problems (See section 1.2.3).

1.2.3. Criticism of the dilution method

Increased popularity of the dilution method lead to closer inspections of various aspects of the method.

Reports on uninterpretable results such as insignificant slopes ($p > 0.05$) or positive slopes in the regression analysis are not uncommon (e.g. Caron et al. 2000, Kim et al. 2007). These failed interpretations occurred in 6 – 74% of the experiments (Kamiyama, 1994, Gifford et al. 1995, Landry et al. 1995, Reckermann & Veldhuis 1997, Lessard and Murrell 1998, Murrell & Hollibaugh 1998, Caron & Dennett 1999, Gaul et al. 1999, Kuipers & Witte 1999, Caron et al. 2000, summarized by Dolan & McKeon 2005), and are likely due to difficulties in detecting low grazing rates with slight slope using small n values. Low grazing rates also requires the difficult detection of slight differences in the initial and final chlorophyll concentrations in highly diluted incubations (Dolan & McKeon 2005). Violations of the method's assumptions may also contribute to failed interpretations (Dolan 2000, Calbet & Landry 2004).

Suspicions on the violation of assumption 3 were often raised. It was found that grazing may saturate at high prey densities, and that grazers functional response curve are not necessarily linear (Gallegos 1989, Evans and Paranjape 1992). In response to these criticisms, Landry et al. (1995) came up with a new approach that combines the dilution method with the fluorescently labeled bacteria (FLB) method of Sherr et al. (1987), which by the use of FLB, allows the independent estimation of the relative grazing rate D, and abolishes the need for assumption 3. Landry et al. (1995) found that the grazing rates estimated from the new approach were essentially identical to those derived from conventional dilution experiments, demonstrating the robustness of conventional dilution experiments.

Regarding the addition of excess nutrients to satisfy assumption 1, Gifford

(1988) found that the action may cause the loss of oligotrich ciliates, but Gallegos (1989) argued that the close coupling of grazing and growth indicates that grazing was not greatly impaired by nutrient addition.

Waterhouse and Welschmeyer (1995) found that compared with grazing rate estimates from cell counts, the grazing rate estimates from pigments, both chlorophyll *a* and carotenoid pigments, were lower. They suggested that the underestimation may be due to incompletely degraded pigments that were ingested when the experiment terminated. On the other hand, Gallegos (1989) found that due to differences of prey concentration in different dilutions, there may be higher grazer growth in low dilutions and higher grazer mortality in high dilutions. The combined effects of such may result in an over-estimation of grazing rates. Dolan et al. (2000) further inspected the effect of dilution on different grazer growth rates and found that different types of grazers may have different growth rates in different dilutions, so they advertise the need to examine the grazers in the experiments to provide information on the grazer populations. They also supported that over-estimation by the dilution method may be common, especially in low chlorophyll waters. Dolan and McKeon (2005) further elaborated on this point by comparing ciliate grazing rates from various studies with a maximum filtration rate for ciliates. They stated that grazing rates from experiments with low chlorophyll were overestimated, and had inflated the overall average grazing rate.

In cases where the dilution method was used to study grazing impact on bacteria, it was found that bacteria were difficult to remove completely from the supposedly particle free seawater, and might thus cause problems in the interpretation of grazing results on bacteria. Li & Dickie (1985) suggested that 0.22 μm ester membranes are most able to remove bacteria from the supposedly particle free seawater. Li (1990) also found that ultraphytoplankton cells were able to pass

through filters into the supposedly particle free seawater, but experimental results showed that dilution experiments were not strongly influenced by these cells. A more important problem with the use of the dilution method on microzooplankton bacterivory was that incubation may significantly alter the community composition of baterioplankton, and therefore does not provide *in situ* grazing estimations (Fuchs et al. 2000).

The many criticisms of the dilution method in no way condemn its use. Instead they serve to inform researchers the need to interpret the results obtained carefully. The ever increasing popularity of its use serves to illustrate this point.

1.2.4. Results of the dilution experiments and their implications

Calbet and Landry (2004) had recently reviewed results of dilution experiments from 66 studies conducted in different climates and ecosystems (Table 1.3). g/μ ratios were transformed to their arctangent values to reduce the impact of large ratios during data analyses. From the analysis of these data, the authors demonstrated the importance of microzooplankton grazers, which removes on average ~ 60% of phytoplankton production in coastal and estuarine systems and ~ 70% in open oceans. Yet Dolan and McKeon (2005) later argued that the results were overestimated and should not exceed 50%, and even lower in oligotrophic systems.

Nevertheless, results from dilution experiments continue to provide insights on the importance of microzooplankton grazing. Especially in relatively more productive waters (e.g. coastal or estuarine systems) where it is traditionally considered that mesozooplankton are the more dominant grazers (Calbet 2001). The efficiency of microzooplankton may be due to their high growth rates and thus quick responses to environmental changes which eventually leading to a close coupling of their grazing and phytoplankton growth (e.g. Suzuki et al. 2002, Safi et al. 2007). It

Table 1.3. Summary of the data used in Landry and Calbet (2004) for the analyses of the global microzooplankton grazing rates. Regions are distiniguished into oceanic (O), coastal (C) and estuarine (E). Climatic characteristics are distiniguished into tropical/subtropical (TR), temperate/subpolar (T), and polar (P) (Modified from Landry & Calbet 2004).

μ	g	Arctan (g:μ)	Zone	Climate	Region	Reference
0.15 – 0.62	0.01 – 0.28	0.066 – 0.660	C	T	North Atlantic Ocean	Landry & Hassett 1982
1.58	0.35	0.218	C	T	North Pacific Ocean	Landry et al. 1984
0.16 – 0.35	0.36 – 1.04	0.799 – 1.246	C	T	North Atlantic	Burkill et al. 1987
0.01 – 0.34	0.02 – 0.17	0.324 – 0.978	E	T	Arctic Ocean	Paranjape 1987
0.24 – 1.68	0.02 – 0.69	0.100 – 0.785	E	T	North Atlantic Ocean	Gifford 1988
0.48 – 2.30	0.41 – 1.60	0.590 – 0.831	E	T	North Atlantic Ocean	Gallegos 1989
0.41 – 1.60	0.02 – 1.08	0.049 – 0.594	C	T	North Atlantic Ocean	Andersen et al. 1991
0.24 – 0.67	0.05 – 0.31	0.160 – 0.644	O	T	North Pacific Ocean	Strom & Welschmeyer 1991
0.03 – 2.15	0.00 – 1.60	0.000 – 1.360	E	T	North Atlantic Ocean	McManus & Ederington-Cantrell 1992
0.37 – 0.67	0.43 – 0.56	0.696 – 0.860	C	T	North Atlantic Ocean	Verity & Vernet 1992
0.01 – 0.72	0.12 – 0.71	0.282 – 1.488	O	T	North Pacific Ocean	Landry et al. 1993
0.01 – 0.97	0.21 – 1.09	0.269 – 1.562	O	T	North Atlantic Ocean	Verity et al. 1993
0.01 – 1.58	0.00 – 0.88	0.000 – 1.512	C	T	North Pacific Ocean	Neuer and Cowles 1994

Table 1.3. (Continued)

μ	g	Arctan (g:μ)	Zone	Climate	Region	Reference
N.A.	0.15 – 0.40	0.738 – 0.815	O	P	Southern Ocean	Burkill et al. 1995
0.46 – 2.14	0.32 – 2.11	0.598 – 0.964	E	TR	North Atlantic Ocean	Dagg 1995
0.01 – 0.63	0.07 – 0.24	0.308 – 1.494	O	T	North Atlantic Ocean	Gifford et al. 1995
0.55 – 1.13	0.39 – 0.57	0.426 – 0.738	O	TR	Equatorial Pacific Ocean	Landry et al. 1995a
0.09 – 1.46	0.00 – 1.67	0.000 – 1.198	O	TR	Equatorial Pacific Ocean	Landry et al. 1995b
0.07 – 1.32	0.00 – 0.66	0.000 – 0.626	O	T	South Atlantic Ocean	Froneman et al. 1996
0.24 – 1.87	0.00 – 0.58	0.000 – 0.504	O	P/T	Southern Ocean/South Atlantic Ocean	Froneman & Perissinotto 1996a
0.45 – 1.48	0.28 – 0.70	0.383 – 0.666	O	T	Southern Ocean	Froneman & Perissinotto 1996b
0.68 – 2.22	0.00 – 0.67	0.000 – 0.479	C	TR	North Atlantic Ocean	Strom & Strom 1996
0.17 – 0.65	0.15 – 0.97	0.723 – 1.096	O	TR	North Atlantic Ocean	Verity et al. 1996
0.01 – 0.09	0.01 – 0.07	0.261 – 1.217	O	P	Southern Ocean	Froneman et al. 1997
0.01 – 0.12	0.01 – 0.07	0.215 – 0.724	E	T	Indian Ocean	Froneman & McQuaid 1997
0.40 – 1.12	0.04 – 1.19	0.070 – 0.813	O	TR	Arabian Sea	Reckermann & Veldhius 1997
0.01 – 0.81	0.00 – 1.01	0.000 – 1.460	C	T	North Atlantic Ocean	Tamigneaux et al. 1997
0.01 – 1.16	0.00 – 0.69	0.000 – 1.326	C	P	Southern Ocean	Tsuda & Kawaguchi 1997
0.25 – 1.06	0.39 – 1.31	0.662 – 1.220	E	TR	Indian Ocean	Ayukai & Miller 1998
0.10 – 0.19	0.01 – 0.07	0.083 – 0.611	O	T	Southern Ocean	Froneman & Balarin 1998
0.01 – 1.55	0.11– 2.51	0.221 – 1.480	O/C	T	South Pacific Ocean	James & Hall 1998

Table 1.3. (Continued)

μ	g	Arctan (g:μ)	Zone	Climate	Region	Reference
0.01 – 2.68	0.16 – 1.32	0.298 – 1.508	O/C	TR	Arabian Sea	Landry et al. 1998
0.08 – 0.75	0.28 – 0.83	0.669 – 1.412	O	TR	North Atlantic Ocean	Lessard & Murrell 1998
0.03 – 1.23	0.00 – 1.19	0.000 – 1.446	E	T	North Pacific Ocean	Murrell & Hollibaugh 1998
0.20 – 3.23	0.00 – 2.22	0.000 – 0.614	E	T	North Atlantic Ocean	Ruiz et al. 1998
0.02 – 1.44	0.11 – 0.82	0.168 – 1.466	O	TR	Arabian Sea	Caron & Denett 1999
0.25 – 1.77	0.11 – 0.68	0.226 – 1.206	O	TR	Arabian Sea	Edwards et al. 1999
0.38 – 0.63	0.08 – 0.47	0.159 – 0.754	O	T	North Atlantic Ocean	Gaul et al. 1999
0.01 – 0.64	0.00 – 0.27	0.000 – 0.810	O	T	North Pacific Ocean	Rivkin et al. 1999
N.A.	N.A.	0.334 – 0.528	E	TR	North Atlantic Ocean	Dolan et al. 2000
0.52 – 1.81	0.20 – 1.26	0.323 – 0.916	O	TR	Equatorial Pacific Ocean	Landry et al. 2000
0.01 – 0.75	0.00 – 0.98	0.000 – 1.166	C	T	North Atlantic Ocean	Putland 2000
0.02 – 0.06	0.00 – 0.02	0.011 – 0.494	E	T	North Atlantic Ocean	Sautour et al. 2000
0.22 – 0.62	0.09 – 0.38	0.189 – 1.034	C	T	North Pacific Ocean	Shinada et al. 2000
0.01 – 0.66	0.00 – 0.45	0.000 – 1.531	C/O	T	North Atlantic Ocean	Wolfe et al. 2000
0.01 – 0.63	0.13 – 0.57	0.695 – 1.495	E/O	T	North Pacific Ocean	Zhang & Wang 2000
0.19 – 0.87	0.17 – 0.75	0.400 – 1.249	C/O	T	North Atlantic Ocean	Archer et al. 2001
0.17 – 1.71	0.51 – 1.78	0.673 – 1.410	O	T	North Atlantic Ocean	Fileman & Burkill 2001
0.01 – 1.90	0.10 – 2.00	0.663 – 1.559	C	TR	North Pacific Ocean	Garcia-Pámares & Lara-Lara 2001

Table 1.3. (Continued)

μ	g	Arctan (g:μ)	Zone	Climate	Region	Reference
0.24 – 1.38	0.06 – 1.59	0.139 – 0.856	O	T	North Atlantic Ocean	Gaul & Anita 2001
0.08 – 0.25	0.10 – 0.23	0.464 – 1.019	O	P	Southern Ocean	Landry et al. 2001
0.07 – 1.07	0.16 – 0.65	0.108 – 1.043	O	TR	North Atlantic Ocean	Quevedo & Anadón 2001
0.16 – 1.00	0.03 – 0.51	0.207 – 0.625	O	P	Southern Ocean	Selph et al. 2001
0.09 – 2.69	0.00 – 1.48	0.251 – 1.074	C	T	North Pacific Ocean	Strom et al. 2001
0.01 – 1.08	0.00 – 0.66	0.000 – 0.549	O	T	North Pacific Ocean	Zhang et al. 2001
0.01 – 0.16	0.94 – 1.03	1.417 – 1.560	C	T	North Atlantic Ocean	Fileman et al. 2002
0.07 – 0.42	0.02 – 0.24	0.224 – 1.068	O	P	Southern Ocean	Landry et al. 2002
0.01 – 0.57	0.08 – 0.72	0.415 – 1.529	O	T	North Pacific Ocean	Liu et al. 2002
0.33 – 1.66	0.08 – 1.25	0.309 – 0.682	E	T	North Atlantic Ocean	Murrell et al. 2002
0.38 – 0.70	0.15 – 0.80	0.305 – 1.096	O	T	North Pacific Ocean	Obayashi & Tanoue 2002
0.53	0.65	0.893	E	T	Arabian Sea	Redden et al. 2002
0.14 – 0.74	0.00 – 0.61	0.000 – 0.833	C	T	North Pacific Ocean	Zhang et al. 2002
0.16 – 1.02	0.04 – 0.73	0.197 – 0.727	O	TR	Equatorial Pacific Ocean	Landry et al. 2003

may also be an indication of an even higher capability than generally appreciated of microzooplankton to consume large phytoplankton, such as diatoms chains, that dominant productive waters (Calbet & Landry 2004). Mesozooplankton however still affects phytoplankton stocks indirectly as predators of microzooplankton (Miller et al. 1995, Buskey et al. 2003).

1.3. The roles of microzooplankton in marine food webs

With their high grazing intensity (See section 1.2.4), it is obvious that microzooplankton plays an important role in marine food webs.

There are increasing evidence of mesozooplankton preference for microzooplankton due to their optimal size, nutritional values and swimming behavior (e.g. Gasparini et al. 2000, Henjes et al. 2007, Calbet 2008). Microzooplankton can therefore be a potentially important intermediate that passes production to mesozooplankton. But this only applies where mesozooplankton can directly exploit the primary micrograzers (Landry & Calbet 2004). In ecosystems such as the oligotrophic subtropical waters near Hawaii, it was found that small micrograzers and long trophic pathways decreased the efficiency of energy transfer to higher trophic levels (Calbet & Landry 1999).

Another often investigated role of microzooplankton is their role in nutrient recycling. Although microzooplankton have high feeding plasticity (See section 1.1), they are generally considered to feed on smaller phytoplankton (e.g. Burkill et al. 1995, Froneman & McQuaid 1997, Palomares-Garcia et al. 2006, Calbet 2008). Ciliates in particular feed optimally on prey that are ~ 8 – 10X smaller than themselves, even though their size ratio between predator and prey can range from 1:1 to 30:1 (summarized by Hansen et al. 1994). This is possibly due to an increase in handling time and a decrease in feeding efficiency when prey size is not optimal,

leading to an energetic cost (Peters 1994). Such preference may have implication on nutrients recycling. Since selectivity grazing towards smaller phytoplankton will leave the faster sinking larger phytoplankton ungrazed, facilitating the export of these phytoplankters along with the nutrients they contain to the sea bottom (Safi et al. 2007). On the other hand, microzooplankton fecal pellets are either unconsolidated or slow-sinking (Buck & Newton 1995), so that nutrient regeneration from these fecal pellets is possible in the euphotic zone (Sieburth et al. 1978). Whether nutrients are mostly exported or regenerated seems to be dependent on the size of the phytoplankton and the size selectivity of the microzooplankton which are dominant in the ecosystem.

With their high grazing impact on phytoplankton, microzooplankton has the ability to strongly impact the phytoplankton community. The requirements to affect phytoplankton blooms significantly include: high abundance; the ability to coincide with algae both in time and in space; and the ability to feed on algae efficiently (Calbet 2008). Of all grazers on phytoplankton, microzooplankton seems to fit these requirements best. It is hypothesized that phytoplankton blooms can only be formed when there is a 'loophole' in microzooplankton grazing, such as when certain species develop defences against microzooplankton (e.g. spines or toxicity), to allow bloom initiation. Subsequent bloom development also depends on decreased microzooplankton grazing (e.g. avoidance of microzooplankton grazing on unfavorable blooming algal species or increase predation of microzooplankton by mesozooplankton) (Irigoien et al. 2005). Although it has been found that both ciliates (e.g. Stom & Welschmeyer 1991, Verity et al. 1991) and dinoflagellates (e.g. Buskey 1997) have chemosensory mechanisms, there is yet detailed information on microzooplankton selectivity. Despite the use of dilution experiments with HPLC to study microzooplankton selectivity towards phytoplankton, the general conclusion is

that microzooplankton has higher grazing rates on phytoplankton groups with higher growth rates (e.g. Burkill et al. 1987, Gaul & Antia 2001).

1.4. Phytoplankton

Phytoplankters are a vast group of photoautotrophic plankton. Common groups of phytoplankton include Bacillariophyceae (Diatoms) and Prymnesiophyceae (Prymnesiophytes). Although the term 'primary producers' commonly associated with phytoplankton implies exclusive photoautotrophy, Dinophyceae (Dinoflagellates) with half of its members being heterotrophic (Jeffrey & Vesk 1997), is also generally considered as a common group of phytoplankton.

Oceanic net primary production can contribute as much as terrestrial production (Oceanic: 46.2%; Terrestrial: 53.8%), and only 2.1% of oceanic production is attributed to macrophytes, with the rest coming from phytoplankton (Field et al. 1998). This demonstrates the importance of phytoplankton as primary producers, acting as the base of all food webs in the world's oceans.

1.4.1. Size classification

The size range of phytoplankton can be large, with the smallest ultraphytoplankton < 0.2 μm (Li 1990) and the largest phytoplankton colonies > 1 cm (Veldhuis et al. 2005). Due to such difference, it is more meaningful to divide phytoplankton into different size fractions: picophytoplankton (0.2 – 2 μm), nanophytoplankton (2 – 20 μm) and microphytoplankton (20 – 200 μm) (Jeffrey & Vesk 1997), for the sake of distinguishing their different roles in different trophic pathways.

1.4.2. Chemotaxonomic marker pigments

Photoautotrophs depend on pigments such as chlorophylls and carotenoids for the absorbance of light energy. These pigments can be extracted and analyzed by high performance chromatography (HPLC). Since some pigments are unique or restricted to certain groups of phytoplankton, they can be used as chemotaxonomic markers for such groups. Table 1.4 shows the list of accessory pigments found in phytoplankton and table 1.5 shows the pigments that can be used as chemotaxonomic markers. Due to the high demand of time and taxonomic skills needed to identify phytoplankton (Jeffrey & Vesk 1997), chemotaxonomic markers provide an efficient and unambiguous mean for phytoplankton identification. Table 1.6 shows the list of the pigments used in this study as chemotaxnomic markers for specific groups of phytoplankton.

1.4.3. Nutrients and phytoplankton dynamics

Besides temperature and irradiance, an important parameter affecting phytoplankton growth and therefore primary production is nutrient contents (Nybakken & Bertness 2004). The type and amount of nutrients present in the ecosystem determines the type of phytoplankton dominating in that system (Table 1.7). One advantage that smaller phytoplankton has in oligotrophic waters is that their larger surface area:volume ratio provides a faster diffusion rate for nutrient uptake, which leads to higher growth rates (Reynolds 2006). It is also demonstrated through the use of two different size phytoplankton, *Ditylum brightwellii* (30 μm diameter) and *Coccolithus huxleyi* (5 μm diameter) that large and small phytoplankton cells differ in sinking rates and Michaelis-Menten constants characteristic of nutrient and light response, thus affecting the nutrient uptake rate. This difference leads to the difference in growth rates among phytoplankton of

Table 1.4. Distribution of major and taxonomically significant pigments in algal divisions/classes. 3 = major pigment (> 10%); 2 = minor pigment (1 – 10%); 1 = rare pigment (< 1%) of the total chlorophylls or carotenoids (Modified from Jeffrey & Vesk 1997).

	Algal Division/Class												
Pigment	**Cyanophyta**	**Prochlorophyta**	**Rhodophyta**	**Cryptophyta**	**Chlorophyceae**	**Prasinophyceae**	**Euglenophyta**	**Eustigmatophyta**	**Bacillariophyta**	**Dinophyta**	**Prymnesiophyceae**	**Chrysophyceae**	**Raphidophyceae**
Chlorophylls													
Chlorophyll *a*	3		3	3	3	3	3	3	3	3	3	3	3
Chlorophyll *b*					3	3	3						
Chlorophyll c_1									3		3		3
Chlorophyll c_2				3					3	3	3	3	3
Chlorophyll c_3											3	3	
Carotenes													
Carotene α		2	2	2	1	1					1		
Carotene β	2	2			2	2	2	2	1	1	1	1	2
Carotene γ					1								
Carotene ε				1								1	
Lycopene				1									
Xanthophylls													
Alloxanthin				3									
Antheraxan-thin					1	1	1						
19-but-fuco-xanthin											3	3	
Crocoxanthin				1									

Table 1.4. (Continued)

Pigment	Algal Division/Class												
	Cyanophyta	Prochlorophyta	Rhodophyta	Cryptophyta	Chlorophyceae	Prasinophyceae	Euglenophyta	Eustigmatophyta	Bacillariophyta	Dinophyta	Prymnesiophyceae	Chrysophyceae	Raphidophyceae
Xanthophylls													
Diadinoxanthin							3		3	3	3	3	3
Diatoxanthin							1		1	1	1	1	1
Dinoxanthin										2			
Fucoxanthin									3		3	3	3
19-hex-fucoxanthin											3		
Lutein					3	2							
Monadoxanthin				1									
Neoxanthin					3	3	1						
Peridinin										3			
Peridininol										1			
Prasinoxanthin						3							
Pyrrhoxanthin										1			
Vaucheriaxanthin ester								2					
Violaxanthin					3	3		3					
Zeaxanthin	3	3	3		2			1					
Biliproteins													
Allophycocyanins	3		3										
Phycocyanins	3		1	3									
Phycoerythrins	3		3	3									

Table 1.5. Summary of signature pigments useful as markers of algal groups (Modified from Jeffrey 1997).

Pigment	Algal group
Chlorophylls	
Chlorophyll *a*	All photosynthetic microalgae (except prochlorophytes)
Chlorophyll *b*	Green algae: chlorophytes, prasinophytes, euglenophtes
Chlorophyll *c* family	Chromophyte algae
Chlorophyll c_1	Diatoms, some prymnesiophtes
Chlorophyll c_2	Most diatoms, dinoflagellates, prymnesiophytes, raphidophytes, cryptophytes
Chlorophyll c_3	Some prymnesiophytes, one chrysophyte, several diatoms and dinoflagellates
Carotenoids	
Carotene α	Cryptophytes, prochlorophytes, rhodophytes, green algae
Carotene β	All algae except cryptophytes and rhodophytes
Xanthophylls	
Alloxanthin	Cryptophytes
19-but-fucoxanthin	Some prymnesiophytes, one chrysophyte, several dinoflagellates
Crocoxanthin	Cryptophyte (minor pigment)
Diadinoxanthin	Diatoms, dinoflagellates, prymnesiophytes, chrysophytes, raphidophytes, euglenophytes
Dinoxanthin	Dinoflagellates
Fucoxanthin	Diatoms, prymnesiophytes, chrysophytes, raphidophytes, several dinoflagellates
19-hex-fucoxanthin	Prymnesiophytes, several dinoflagellates
Lutein	Green algae: chlorophytes, prasinophytes
Monadoxanthin	Cryptophytes (minor pigment)
Peridinin	Dinoflagellates
Prasinoxanthin	Some prasinophytes
Pyrrhoxanthin	Dinoflagellates (minor pigment)
Vaucheriaxanthin ester	Estigmatophytes

Table 1.5. (Continued)

Pigment	Algal group
Xanthophylls	
Violaxanthin	Green algae: chlorophytes, prasinophytes; eustigmatophytes
Zeaxanthin	Cyanophytes, prochlorophytes, rhodophytes, chlorophytes, estigmatophytes (minor pigment)
Biliproteins	
Allophycocyanins	Cyanophytes, rhodophytes
Phycocyanin	Cyanophytes, cryptophytes, rhodophytes (minor pigment)
Phycoerythrin	Cyanophytes, cryptophytes, rhodophytes

Table 1.6. Pigments used as chemotaxonomic markers of specific algal taxa in this study.

Pigment	Algal group
Peridinin	Dinoflagellates
Fucoxanthin	Diatoms
19-hex-fucoxanthin	Prymnesiophytes
Alloxanthin	Cryptophytes
Lutein	Green algae
Zeaxanthin	Cyanobacteria
Chlorophyll *b*	Green algae
Chlorophyll *a*	All phytoplankton

Table 1.7. Effects of nutrient quantity and chemical composition on phytoplankton (Modified from Legendre & Rivkin 2002).

Low nutrients: Small phytoplankton
Intermediate and high nutrients: Large phytoplankton
High Si:N and Si:P ratios: diatoms
Low Si:N and high N:P ratios: dinoflagellates
Edible taxa
Inedible taxa: blooms, followed by sinking to depth of ungrazed phytoplankton
Low Si:N and high P:N ratios: occasional blooms of inedible N_2-fixing filamentous cyanobacteria

different sizes in different environments. It was found that *D. brightwellii*, and generally other species of large phytoplankton would have higher growth rates than small phytoplankton in areas with high light intensity and nutrient concentration (Parsons & Takahashi 1973).

Higher nutrient contents also generally lead to higher chlorophyll contents. Chisholm (1992) stated that it is an unambiguous fact that the proportion of small cells decreases as chlorophyll increases. And this serves as another support of the occurrence of higher proportions of smaller phytoplankton in nutrient poor ecosystems.

1.5. Hypothesis

From the general considerations of higher composition of small phytoplankton in ecosystems with low nutrients (See section 1.4.3) and microzooplankton selectivity towards small phytoplankton (See section 1.3) sprouts the hypothesis that in ecosystems with low nutrient levels, there will be a high composition of small phytoplankton and high microzooplankton grazing rates.

1.6. Objectives

1. To test the hypothesis in section 1.5 that ecosystems with lower nutrient concentrations will have higher compositions of small phytoplankton and higher microzooplankton grazing rates by comparing phytoplankton communities and microzooplankton grazing rates in two bays (Tolo Harbour and Mirs Bay) with different nutrient levels.
2. To study microzooplankton selectivity towards phytoplankton of different taxonomic groups.

3. To study microzooplankton selectivity towards phytoplankton of different size fractions.

1.7 Research outline

1.7.1. Microzooplankton grazing rates and phytoplankton growth rates

The microzooplankton grazing rates and phytoplankton growth rates in Tolo Harbour and Mirs Bay (See section 1.8) were estimated simultaneously using the dilution method (See section 1.2). Dilution experiments were carried out bimonthly during the period of March 2007 to January 2008 to provide seasonal data on microzooplankton grazing rates and phytoplankton growth rates.

1.7.2. Phytoplankton group selection

To study microzooplankton selectivity towards phytoplankton of different taxonomic groups, HPLC was used to analyze phytoplankton chemotaxonomic marker pigments that represented different groups (See section 1.4.2.).

1.7.3. Phytoplankton size selection

To study microzooplankton selectivity towards phytoplankton of different size fractions, the phytoplankton community will be divided into three different size fractions: < 200 μm, < 20 μm, and < 5 μm, using 20 μm and 5 μm meshes just before sample collection.

1.8. Study sites

To test the hypothesis in section 1.5, at least two sites with similar parameters except nutrient contents were needed. Tolo Harbour and Mirs Bay located at the eastern part of Hong Kong are suitable sites for such a study (Figure 1.1).

1.8.1. Tolo Harbour

Tolo Habour is semi-enclosed bay. It consists of a shallow inner basin and a narrow channel that connects it to the more open Mirs Bay. Ever since urban developments of the area around the harbour started in the 1970s, increased sewage discharge, together with the fact that the harbour is poorly flushed due to its landlocked topography, has caused severe eutrophication which eventually leads to the initiation of the Tolo Harbour Effluent Export Scheme in the 1990s to reduce nutrient loading (Lam & Ho 1989). In spite of obvious improvements, productivity (in terms of chlorophyll *a* concentrations) within the harbour remains high (Figure 1.2) and algal blooms still occur frequently (Arega & Lee 2000, HKEPD 2006).

1.8.2. Mirs Bay

As mentioned in the above section, Mirs Bays is connected to Tolo Harbour so that a portion of pollutants from Tolo Habour may be brought to Mirs Bay by tidal currents (Lee & Arega 1999). Like Tolo Harbour, Mirs Bay is also a semi-enclosed bay and is connected to both Tolo Harbour and the South China Sea. Unlike Tolo Harbour, the land surrounding Mirs Bay is sparsely populated, water quality in Mirs Bay is therefore better than Tolo Harbour (HKEPD 2006).

A)

B)

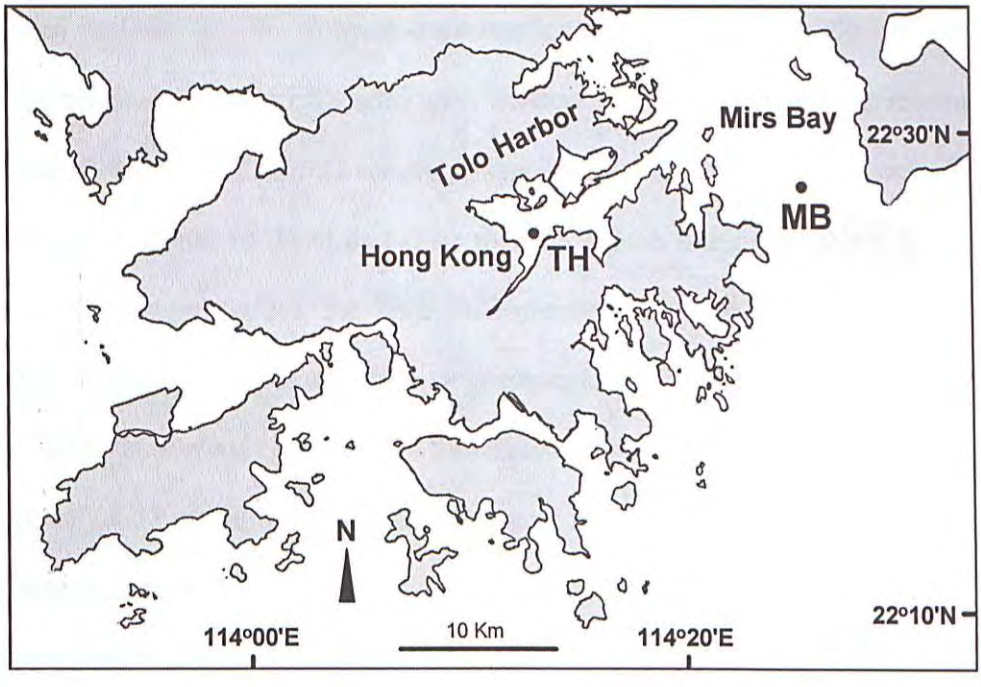
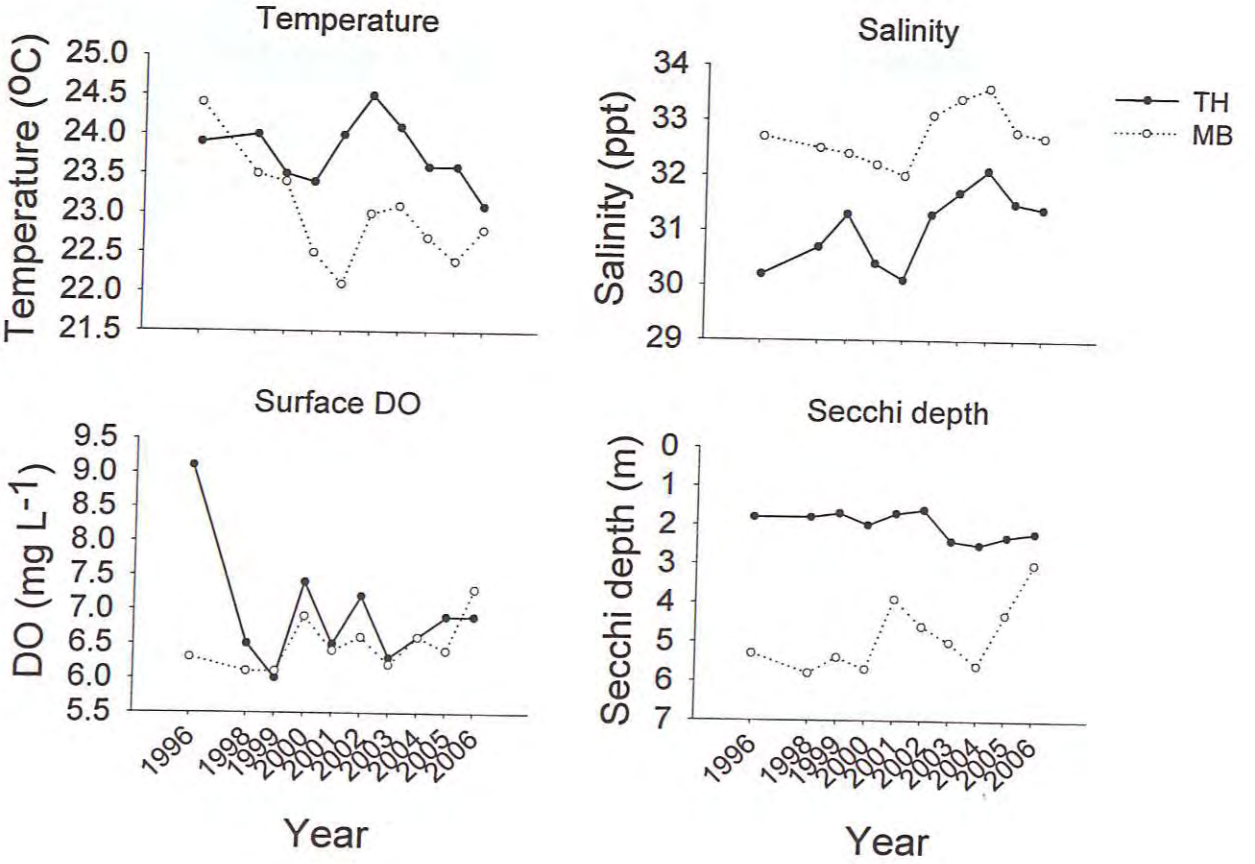


Figure 1.1. A map of China showing the location of Hong Kong (A). A map of Hong Kong showing the location of the study sites TH and MB in Tolo Harbour and Mirs Bay respectively (B).

1.8.3. Biological and physio-chemical parameters

Figure 1.2 shows the annual mean temperature, salinity, dissolved oxygen content (DO), sechi depth, various nutrient concentrations, and chlorophyll *a* contrations of the two designated study sites in Tolo Harbour (TH) and Mirs Bay (MB) (Figure 1.1) from 1996 – 2006. Except for DO, all the other parameters were averages of samples from the water surface, mid-depth, and bottom. DO was from the water surface. Temperature and DO were similar between the two sites. Salinity was expectedly slightly lower in TH due to freshwater inputs from several streams. Secchi depth was lower in TH, indicating a higher turbidity. This may in part be due to the high chlorophyll *a* concentrations, but may also be due to higher amount of suspended particles in TH. Nutrient concentrations were generally higher in TH, although the Tolo Harbour Effluent Export Scheme may have started a decreasing trend, and differences in nutrient concentrations between the two sites are gradually diminishing. It should be noted that since the data shown in Figure 1.2 are annual mean depth-averaged values, the large difference between the two sites may be deflated by events such as stratification or seasonal low points. During the period of 2003 – 2006, the highest concentration for total inorganic nitrogen was 23.56 μM in TH and only 8.57 μM in MB. Also, the maximum values in MB seldom exceeded 5 μM while it always did in TH (HKEPD 2004, 2005, 2006, 2007). Chlorophyll *a* concentrations in TH have also decreased gradually since 1996, most likely as a consequence of lower nutrient loading, but have still remained obviously higher than the chlorophyll *a* concentrations in MB.


Temperature
Temperature (°C)
25.0
24.5
24.0
23.5
23.0
22.5
22.0
21.5
Salinity
Salinity (ppt)
34
33
32
31
30
29
TH
MB
Surface DO
DO (mg L-1)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
1996
1998
1999
2000
2001
2002
2003
2004
2005
2006
Year
Secchi depth
Secchi depth (m)
0
1
2
3
4
5
6
7
1996
1998
1999
2000
2001
2002
2003
2004
2005
2006
Year

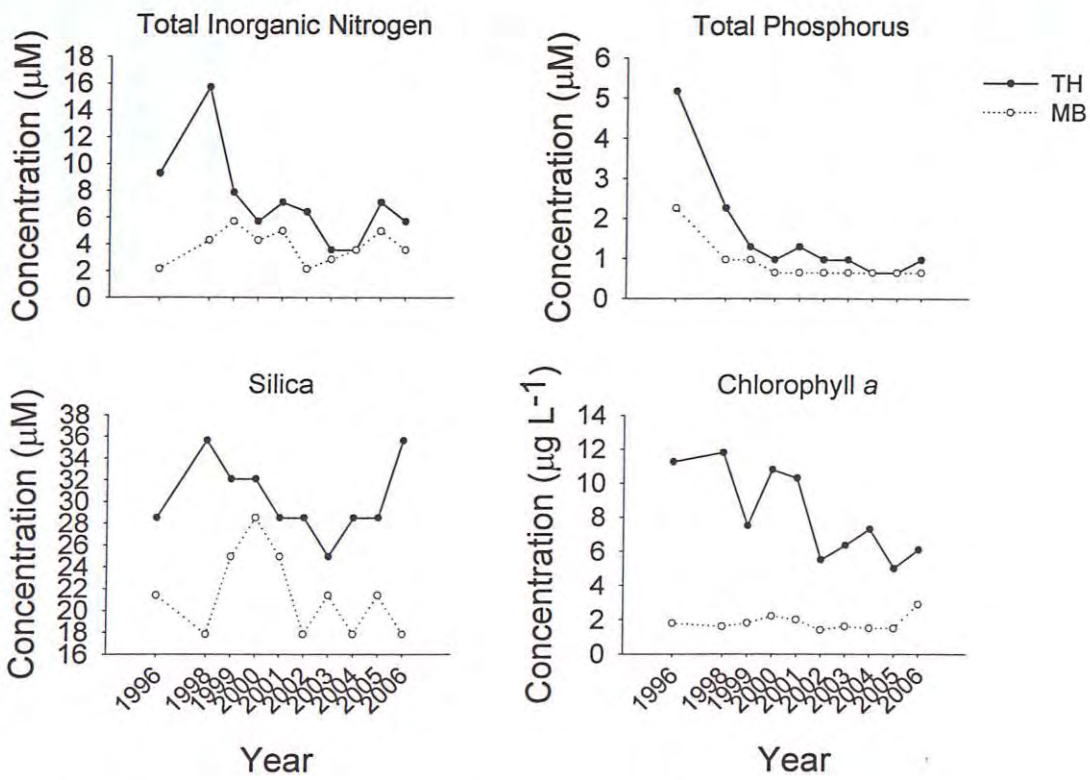


Figure 1.2. Summary of the annual mean depth averaged (except for DO) data of temperature, salinity, surface dissolved oxygen content (DO), secchi depth, total inorganic nitrogen, total phosphorus, silica, and chlorophyll *a* concentrations in TH and MB from 1996 – 2006 (HKEPD 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007).

Chapter 2

Materials and methods

2.1. Study site and sampling

This study was conducted at two sampling sites, one in the inner part of Tolo Harbor (TH) (water depth ~ 8 m), and one in central Mirs Bay (MB) (water depth ~ 22 m) (Figure 1.1). Six sets of dilution experiments were conducted about every other month and on separate dates for each site starting from March 07 to January 08 (Table 2.1). Temperature, salinity and DO at the surface (0.5 m) were measured using a Hydrolab (Hydrolab Corporation). Water transparency was estimated with a Secchi disc. Surface seawater for dilution experiments was collected with a 3 L plastic bucket and filtered through a 200 μm mesh to remove mesozooplankton. The water was returned to the laboratory in large plastic containers (20 L). About 250 ml subsamples of the seawater were filtered through Whatman GF/F glass-fiber filters (0.7 μm pore size, 47 mm diameter) and frozen at -20℃ for later dissolved inorganic nutrients analysis. Nutrient concentrations were analyzed by a SKALAR Continuous Flow Analyzer (SKALAR Analytical).

2.2. Dilution experiments

Particle-free seawater (FSW) was prepared by filtering natural seawater through Millipore 0.22 μm membrane filter. This filtered seawater was used to dilute unfiltered seawater (UFSW) into four different dilutions (25 UFSW:75 FSW, 50 UFSW:50FSW, 75 UFSW:25 FSW and 100 UFSW:0 FSW). Nutrients were added to provide a final concentration of 20 μM NO_3^- and 1 μM PO_4^{2-} to prevent nutrient depletion during incubation (See section 2.2.1). Incubations without nutrient addition (Unenriched incubations) of 100:0 UFSW:FSW were prepared to determine

Table 2.1. Experiment dates for all dilution experiments in TH and MB.

Month	Site	Date
March 07	TH	15 March 2007
	MB	29 March 2007
May 07	TH	3 May 2007
	MB	9 May 2007
August 07	TH	27 August 2007
	MB	30 August 2007
September 07	TH	20 September 2007
	MB	28 September 2007
November 07	TH	8 November 2007
	MB	15 November 2007
January 08	TH	11 January 2008
	MB	17 January 2008

phytoplankton growth rate under ambient nutrient conditions. Dilution experiments were carried out in 1.2 L glass bottles. Duplicates were used for each dilution. The bottles were incubated for 24- or 48 h at the surface (~ 0.5 m) of a large outdoor tank containing natural seawater from Tolo Harbour. Dilution experiments for MB usually lasted 48 h to provide higher final pigment concentrations for HPLC detection.

All apparatus used were washed with 10% HCl, then rinsed with milli-Q H_2O and finally rinsed with FSW prior to the experiments to remove any nutrients adhered on the surface.

2.2.1. Preliminary dilution experiments and enrichment tests

A preliminary dilution experiment was conducted in early December 2006 using the methods described above (with phytoplankton in the < 200 μm and < 5 μm size fractions from Tolo Harbour). Negative growth rates in unenriched incubations indicated that nutrient addition was needed for unlimited phytoplankton growth (See section 1.2) (Table 2.2).

Preliminary enrichment tests were done in late December 2006 with water from TH and MB to estimate the amount of nutrients required to support unlimited phytoplankton growth. The tests involved adding different amounts of nitrogen (as $NaNO_3$) or phosphorus (as K_2PO_4) and comparing the apparent chlorophyll *a* growth rate. The range of nutrients tested were 5 – 30 μM nitrogen and 0.5 – 10 μM phosphorus in TH, and 20 – 150 μM nitrogen and 1 – 50 μM phosphorus in MB. The range tested for MB was higher due to the assumption that seawater from MB contained lower levels of nutrients than seawater from TH. Silica supplements were not considered because data (Figure 1.2) published by the HKEPD indicated that seawater from both sites contained high levels of silica (e.g. ~ 3 – 7X of nitrogen and 15 – 35X of phosphorus in 2006 (HKEPD 2006)). Also because silica was mainly

Table 2.2. Summary of the revised estimated pigment specific phytoplankton growth rate in ambient nutrients (μ_0) and microzooplankton grazing rate (g) of various pigments for the < 200 μm and < 5 μm size fractions in the preliminary dilution experiment in December 2006. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	μ_0	g
< 200 μm	Peri	-0.20	0
	Fuco	0.10	0
	Chl *a*	-0.15	0
< 5 μm	Peri	1.19	-1.34
	Fuco	0.54	0
	Chl *a*	1.24	-1.09

required by diatoms, the addition of silica might shift the composition of the phytoplankton community.

One-way ANOVA analysis of the results (Figures 2.1) showed that the apparent chlorophyll *a* growth rate was not significantly limited by nutrients. But due to the occurrence of negative growth rates in unenriched trial experiments (Table 2.2) and the expectation of higher nutrient requirements during warmer temperatures, it was decided that nutrient supplements should be added. The results of the enrichment tests provided one of the references used to determine the amount of nutrients needed. Other references in the decision included the amounts of nutrients used in previous studies (Table 1.2) and the amount of nutrients present in TH and MB according to HKEPD (Figure 1.2).

20 μM of nitrogen (as $NaNO_3$) and 1 μM of phosphorus (as K_2PO_4) were chosen because these concentrations were similar to the amount of nutrients used by other investigators (Table 1.2) and were comparable to the highest concentrations recorded in TH in 2005 (N: 23.56 μM; P: 0.97 μM) (HKEPD 2006). The same amount of nutrients was added in all dilution experiments to keep factors of the experiments consistent.

2.2.2. HPLC

Seawater sample was filtered through Whatman GF/F glass-fiber filters (0.7 μm pore size, 47 mm diameter). The filters were blotted dry and stored at -80℃ until pigment extraction. To study the microzooplankton grazing rates on different size fractions of phytoplankton, phytoplankton was divided into three size fractions: < 200 μm (total phytoplankton), < 20 μm (nanophytoplankton) and < 5 μm (picophytoplankton). < 20 μm and < 5 μm samples were collected by passing sample seawater through 20 μm and 5 μm meshes respectively before pigments

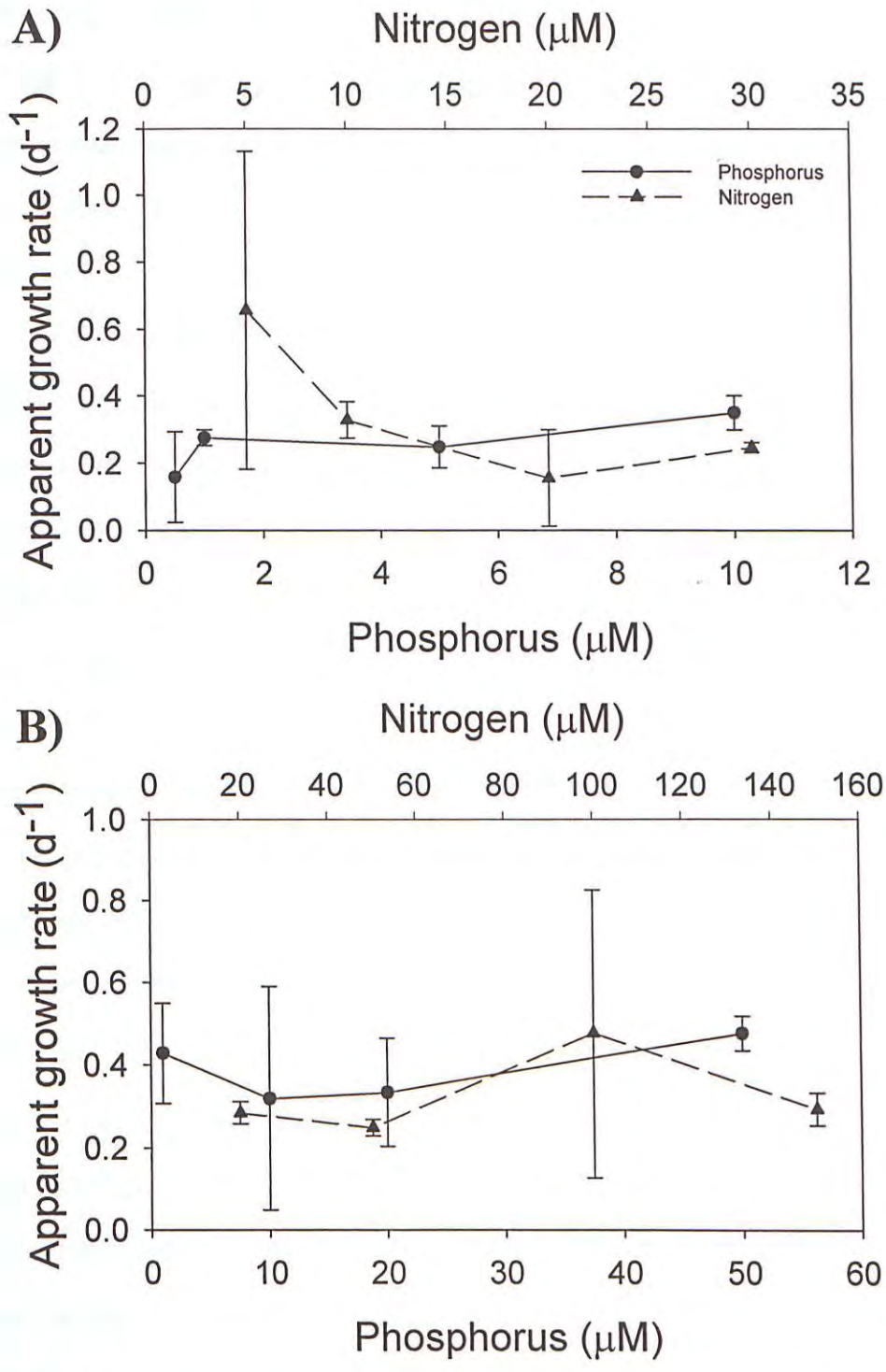


Figure 2.1. The mean apparent chlorophyll *a* growth rates (± standard deviation) after 24-h of incubation with different amount of nitrogen (as $NaNO_3$) or phosphorus (as K_2PO_4) addition in the preliminary enrichment tests conducted in December 2006 for TH (A) and MB (B).

samples were collected on the GF/F filters. Pigment concentrations in the 20 – 200 μm and 5 – 20 μm size fractions were obtained by calculation. By definition, picophytoplankton referes to the 0.2 – 2 μm size range (See section 1.4.1), but < 5 μm was chosen instead because pigment concentrations in MB would be too low for HPLC analysis if < 2 μm were used.

For pigment extraction, the frozen filters are cut into small pieces and 4 ml of 90% HPLC grade acetone was added. The samples were sonicated for 30 min and extracted at 4℃ for 24 h. Extracted samples were centrifuged for 7 min at 4800 rpm (200x g) at 4℃. The supernatant containing pigments was collected using disposable syringes and passed through a NALGENE syringe filter with PTFE membrane (0.2 μm pore size, 13 mm diameter). 20 μl of the extract was injected into a Hewlett Packard HP 1100 series HPLC for analysis. The system consisted of a quaternary pump with online degasser, an injector with injection valve of 20 – 25 μl sample loop, a multi-signal fluorescence detector, and a multi-wavelength UV-VIS detector with a wavelength detection range of 190 – 950 nm. The column used was an Aiglent Eclipse XDB-C18 polymeric reversed phase column (4.6 mm ID x 25 cm, 5 μm particle size) with a flow rate of 1 ml min^{-1}. Three solvents were used for analysis, solvent A was 80:20 HPLC grade methanol:0.5 M ammonium acetate, solvent B was 90:10 HPLC grade acetonitrile:mili-Q water, and solvent C was pure HPLC grade ethyl acetate. Total run time was 30 min. The system program of the solvents is shown in table 2.3. Pigments were detected by the UV/VIS detector set at 436 nm with 385 nm as reference wavelength. Results of the analyses were processed by a Hewlett Packard HPLC ChemStation integrator-processor. Pigment standards (DHI) were used for identification and calibration. Pigments were identified according to the retention time and their concentrations were calculated based on the area of the peaks in the chromatogram.

Table 2.3. The HPLC solvent system program used in all HPLC analyses in this study.

Time (min)	Flow rate ($ml\ min^{-1}$)	% Solvent A	% Solvent B	% Solvent C
0.0	1.0	100	0	0
4.0	1.0	0	100	0
18.0	1.0	0	20	80
21.0	1.0	0	100	0
23.5	1.0	100	0	0
29.0	1.0	100	0	0

2.2.3. Pigment data analysis

When more than three data points (n) were available, linear regression plots of pigment specific apparent growth rate ($1/t \ln(P_t/P_o)$ against the fraction of unfiltered seawater (D) were made (See section 1.2 for details). The y-intercept of the regression curve gives the 'true' growth rate of the pigment in enriched nutrients, or the estimated pigment specific potential phytoplankton growth rate (μ_n). The slope of the regression curve gives the mortality rate of the pigment or estimated pigment specific microzooplankton grazing rate (g), since microzooplankton grazing was assumed to be the major cause of mortality. The significance of the slope was tested (Student's t test, null hypothesis: slope = 0, $p < 0.05$) to show whether the grazing estimate was significant. In the case of positive slopes and insignificant grazing estimates ($p > 0.05$), g was assumed to be 0, and μ_n will be the pigment specific apparent growth rate of the enriched 100:0 UFSW:FSW incubation (Kim et al. 2007). To avoid confusion, the raw data from the linear regression analyses, i.e. the y-intercept and slope of the regression curve, will be referred to as k and m instead of μ_n and g directly. The estimated pigment specific phytoplankton growth rate in ambient nutrient conditions (μ_o) were calculated by subtracting g from the pigment specific apparent growth rate of the unenriched 100:0 UFSW:FSW incubation, i.e.

$$\mu_o = \text{Pigment specific apparent growth rate} - g$$

The percentage of the pigment specific phytoplankton standing stock grazed (SS grazed) was calculated using the equation (Safi et al. 2007):

$$\text{SS grazed} = (1 - e^{-g})\ 100$$

The percentage of the pigment specific phytoplankton production grazed (Production grazed) was calculated using the equation (Safi et al. 2007):

$$\text{Production grazed} = 100\ \frac{(1 - e^{-g})}{(1 - e^{-\mu_0})}$$

2.2.4. Phytoplankton and microzooplankton community analysis

Subsamples (50 - 250 ml) of water from all < 200 μm 100:0 USFW:FSW incubations was preserved in Lugol's solution (2% final concentration) for analysis of phytoplankton and microzooplankton composition and abundance. The samples were kept in dark at 4℃ until analysis. Samples for analysis were concentrated (~ 5X) by sedimentation. Samples were allowed to settle for a week in a measuring cylinder, and the fluid in the upper layer was removed with a pipette. Concentrated samples were transferred to a Sedgwick-Rafter counting chamber and counted under an inverted microscope at 400X (Leica).

Dinoflagellates were identified to the genus level when possible, and grouped into their own group "Dinoflagellates". Diatoms, the most abundant phytoplankton, were also grouped together and identified to the genus level when possible. All other phytoplankton that were not diatoms or dinoflagellates were grouped into "Others", which included unidentified cells and low densities taxa such as cryptophytes and silicoflagellates. Microzooplankters were grouped under "Microzooplankton". Oligotrichs and choreotrichs ciliates were identified as "Ciliates", and tintinnids were identified separately as "Tintinnids".

Chapter 3

Results

3.1. Field parameters

3.1.1. Physiochemical parameters

Surface water temperature and dissolved oxygen content were very similar between both sites (Figure 3.1). Surface water salinity was slightly lower in TH, most probably due to freshwater input into Tolo Harbour from several small streams. Secchi depth, which is an indication of water transparency, was also usually lower in TH. The lower transparency in TH may be due to higher densities of suspended particles and phytoplankton. The presence of suspended particles may also explain the lack of correlation between chlorophyll *a* concentrations and secchi depth.

Surface water nutrient concentrations were also very similar between both sites (Figure 3.2), which was completely unexpected and contradictory to our hypothesis. All four types of nutrients (Ammonia, nitrite and nitrate, silica, and orthophosphorus) measured varied in the same pattern, but did not give any general pattern. The discrepancy of the results with the expectation of higher nutrient concentrations in TH may be due to the fact that the water samples collected in this study's analyses were from the water surface, which may be easily affected by factors such as rain or anthropogenic inputs, while data from the HKEPD presented in Figure 1.1 were depth averaged data from water surface, mid-depth and bottom. Another possible reason for the lack of difference in nutrient concentrations between the two sites may be the success of the Tolo Habour Effluent Scheme in reducing nutrient loading in Tolo Harbour (See section 1.8.1).

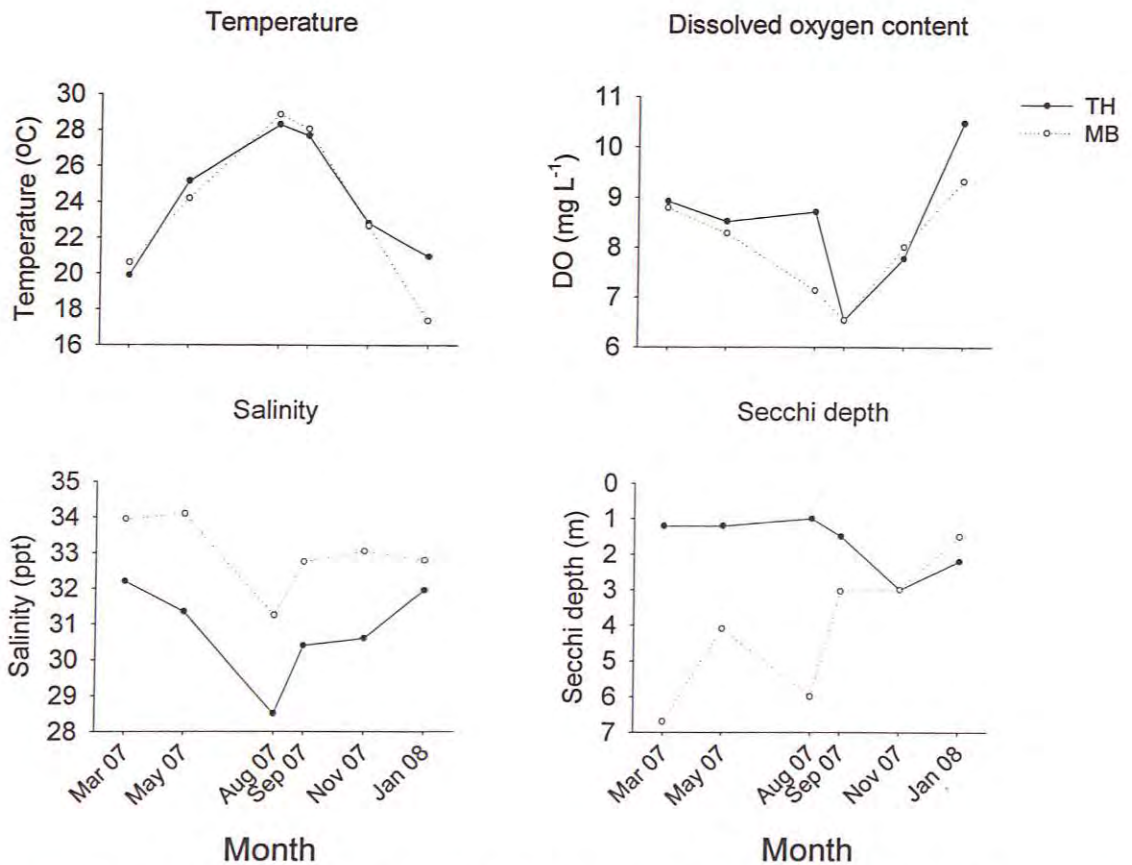


Figure 3.1. Temporal variations in mean temperature, dissolved oxygen, salinity, and secchi depth) in TH and MB during the study period March 07 – January 08. Temperature, dissolved oxygen and salinity were measured at the surface (0.5 m).

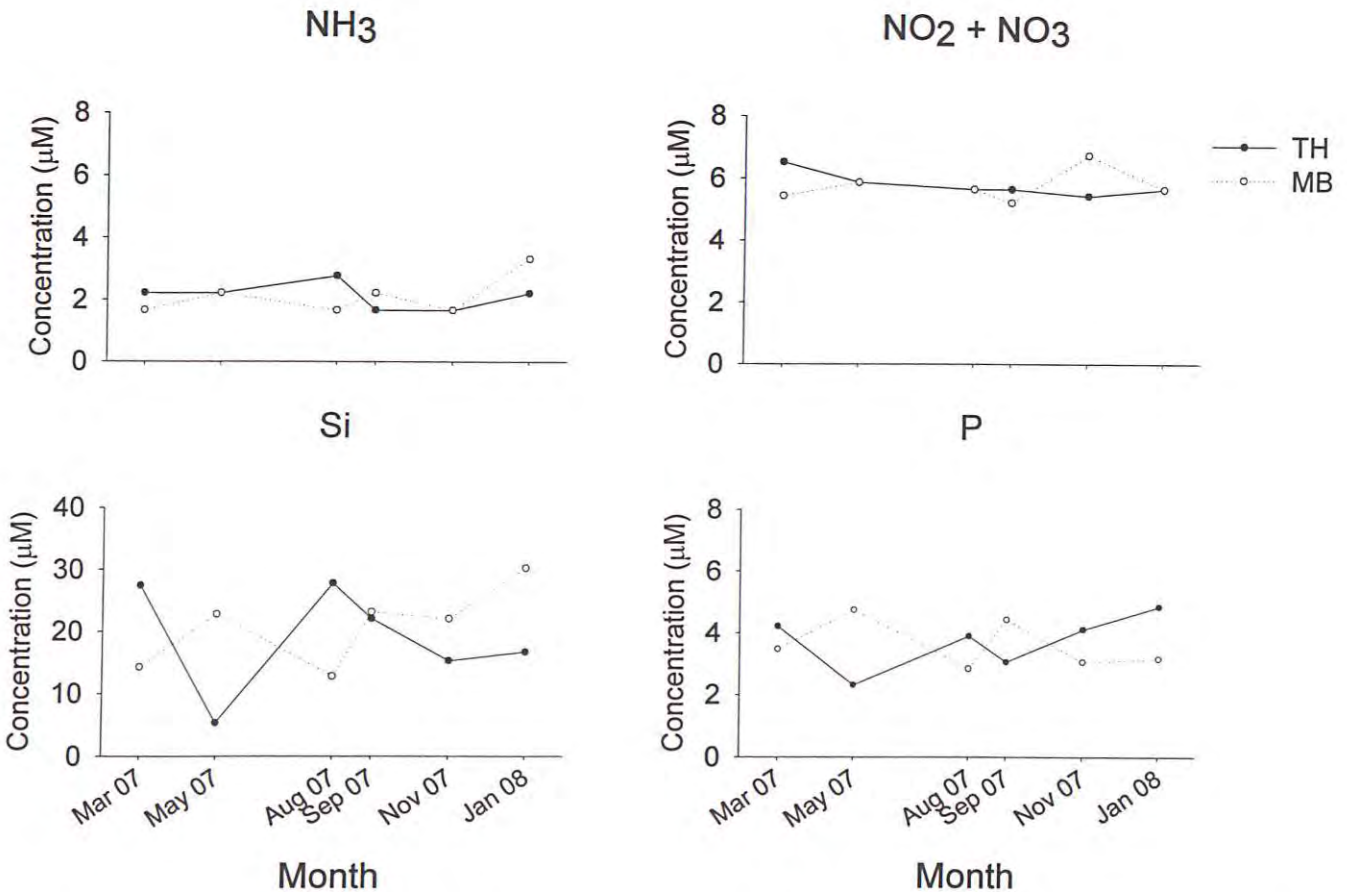


Figure 3.2. Temporal variations in ammonia, nitrite and nitrate nitrogen, silica, and orthophosphate concentrations in TH and MB during the study period March 07 – January 08. Water samples were taken at the surface (0.5 m).

3.1.2. Chlorophyll *a*

Despite the similarity in nutrient concentrations between the two sites, the total surface chlorophyll *a* levels were much higher in TH than in MB (Figure 3.3). And so our hypothesis could still be tested if MB had a higher proportion of smaller size fractions of phytoplankton according to Chisholm's (1992) statement of a higher proportion of small phytoplankton in lower chlorophyll contents ecosystems (See section 1.4.3). Both sites had relatively higher total chlorophyll *a* concentrations during the colder months of March 07 and January 08, and decreased in total chlorophyll *a* concentrations as temperature became higher. TH had the lowest total chlorophyll *a* concentrations throughout the summer months from August to November, but MB total chlorophyll *a* concentrations peaked in September and decreased gradually from then.

A closer look at the size composition of chlorophyll *a* concentrations (Figure 3.4) showed another surprisingly discrepancy from our hypothesis. MB only had higher proportions of small cells during the August and September. And there was no statistical difference (Mann Whitney U test, 95% confidence level) in the percentage compositions of < 5 μm chlorophyll *a* between TH and MB. While < 5 μm phytoplankton was generally the major contributor (> 50%) to chlorophyll *a* in MB except in January 08, TH also had high proportions of < 5 μm phytoplankton except during the warmest two months of August and September, with 15.9% and 33.1% of < 5 μm phytoplankton chlorophyll *a* respectively.

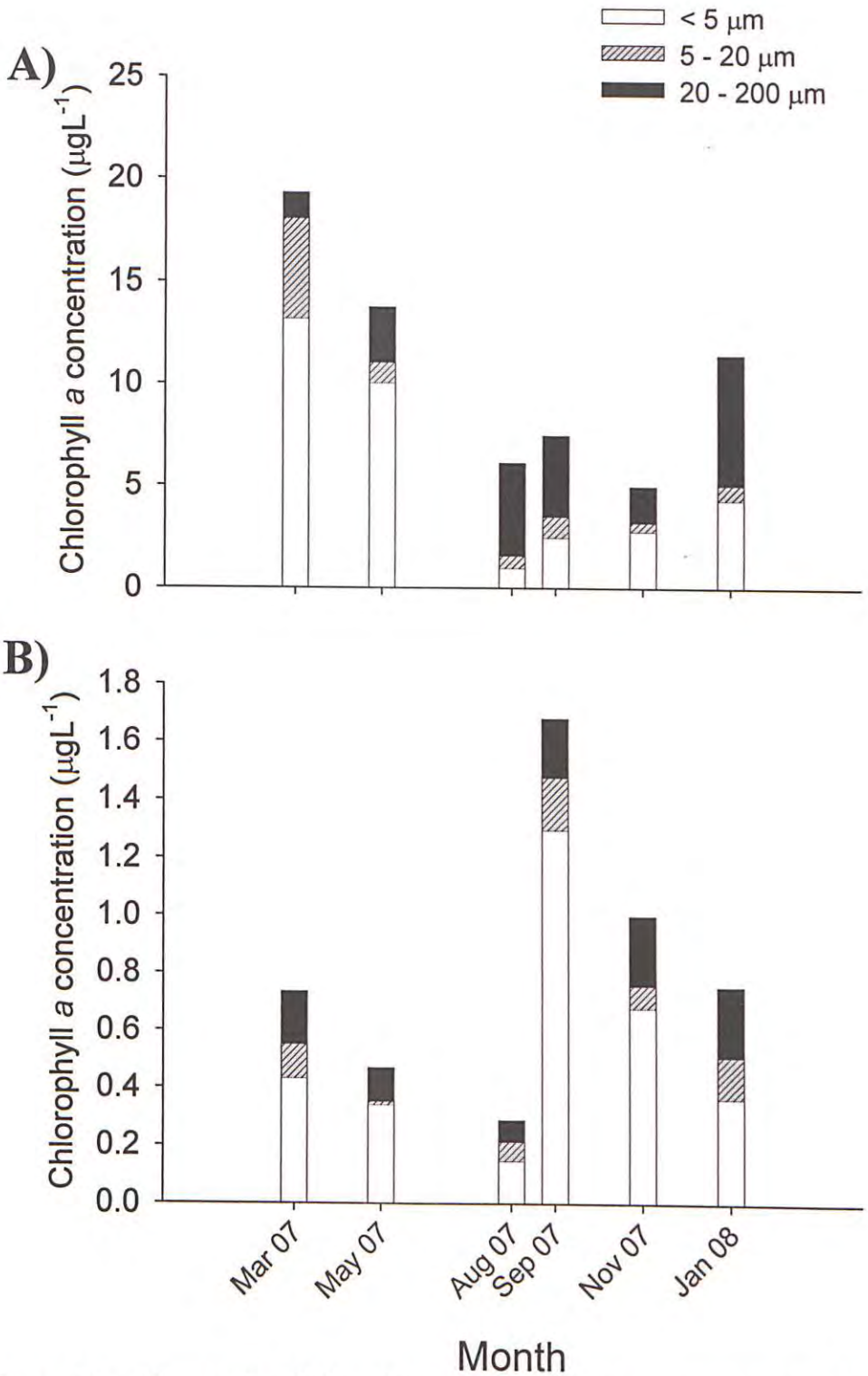


Figure 3.3. Temporal variations in mean chlorophyll *a* concentration of < 5 µm, 5 – 20 µm and 20 – 200 µm phytoplankton in TH (A) and MB (B) during the study period March 07 – January 08.

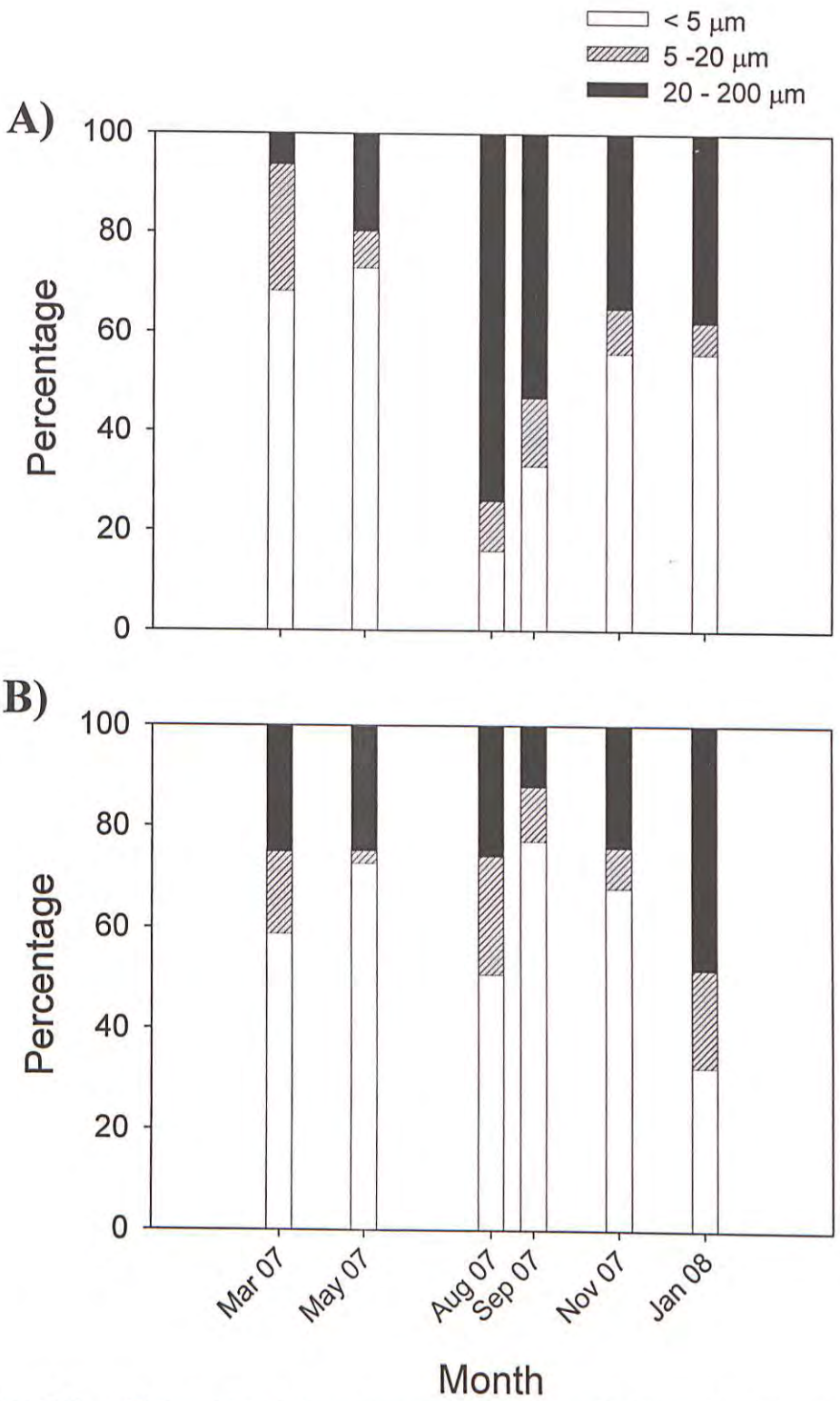


Figure 3.4. Temporal variations in mean percentage of < 5 µm, 5 – 20 µm and 20 – 200 µm chlorophyll *a* in the total chlorophyll *a* concentration in TH (A) and MB (B) during the study period March 07 – January 08.

3.2. Initial conditions

3.2.1. Phytoplankton pigment and size fraction compositions

Our HPLC analyses detected various accessory pigments in addition to the chemotaxonomic markers used in this study (Figures 3.5 – 3.10). Pigment concentrations for the 5 – 20 μm and 20 – 200 μm were deduced by subtracting < 5 μm pigment concentrations from < 20 μm pigment concentrations, and < 20 μm pigment concentrations from < 200 μm pigment concentrations respectively. TH had ~0.5 – 50X higher concentrations of most pigments in all samples than MB. The persistent presence of peridinin, fucoxanthin, 19-hex-fucoxanthin, and alloxanthin indicated the persistent presence of diatoms, dinoflagellates, prymnesiophytes and cryptophytes respectively in both sites. Zeaxanthin, chlorophyll *b* and lutein, representing cyanobacteria and green algae, were often found in both sites as well. Prasinoxanthin, the chemotaxonomic marker for prasinophytes was only found occasionally in MB. It yielded very few significant grazing or growth rates and was therefore not included in further mentioning or analysis in this study. Fucoxanthin had the highest concentration of all accessory pigments in most samples, indicating the dominance of diatoms in both sites.

The composition of different size fractions among the various pigments followed that of chlorophyll *a* generally, with only a higher composition of microphytoplankton (20 – 200 μm) in August and September 07 in TH and in January 08 in MB.

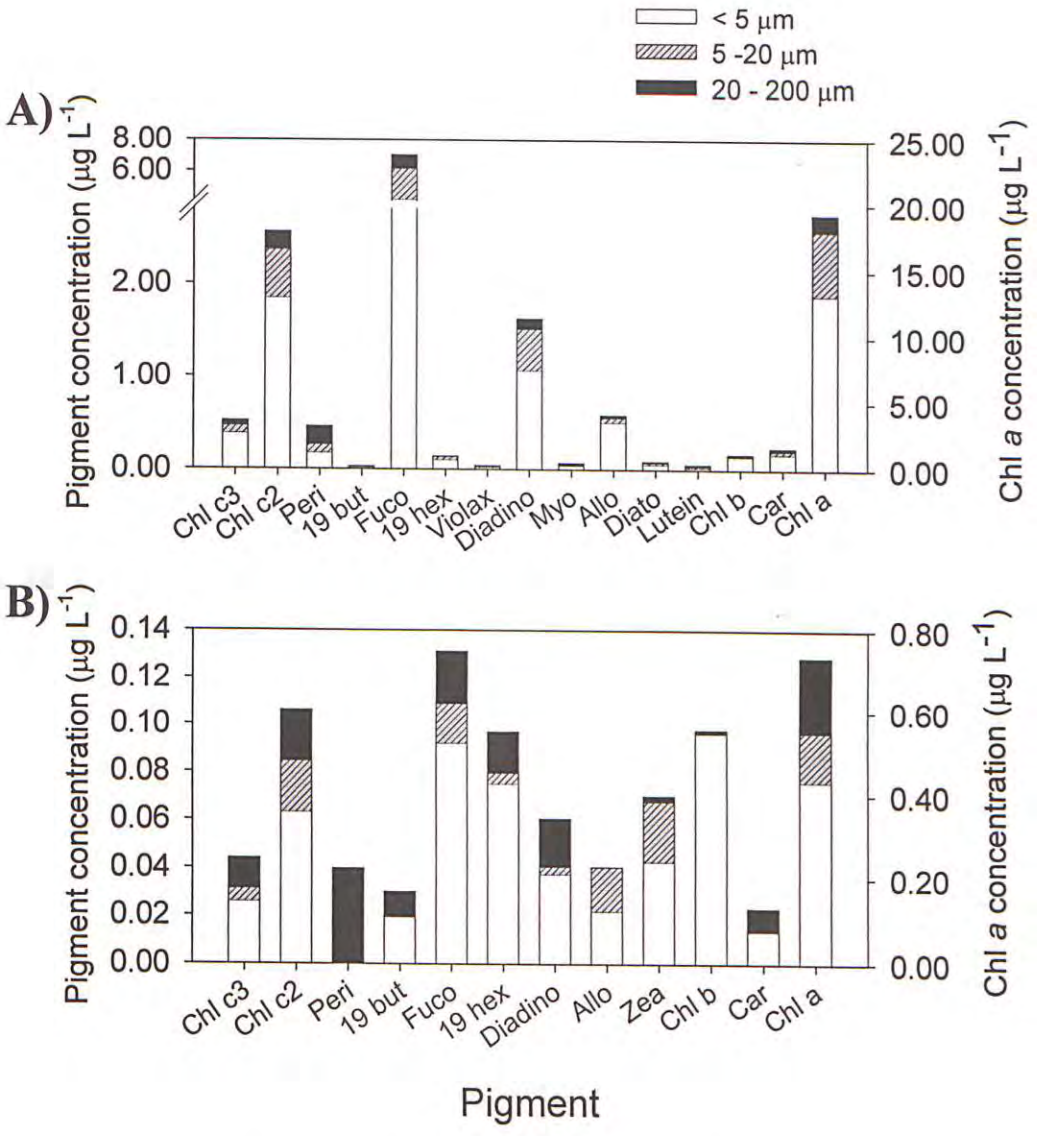


Figure 3.5. Mean concentration of < 5 µm, 5 – 20 µm and 20 – 200 µm phytoplankton pigments in surface seawater collected in TH (A) and MB (B) for dilution experiments in March 07. Refer to table A.1 in Appendix for pigments abbreviations interpretations.

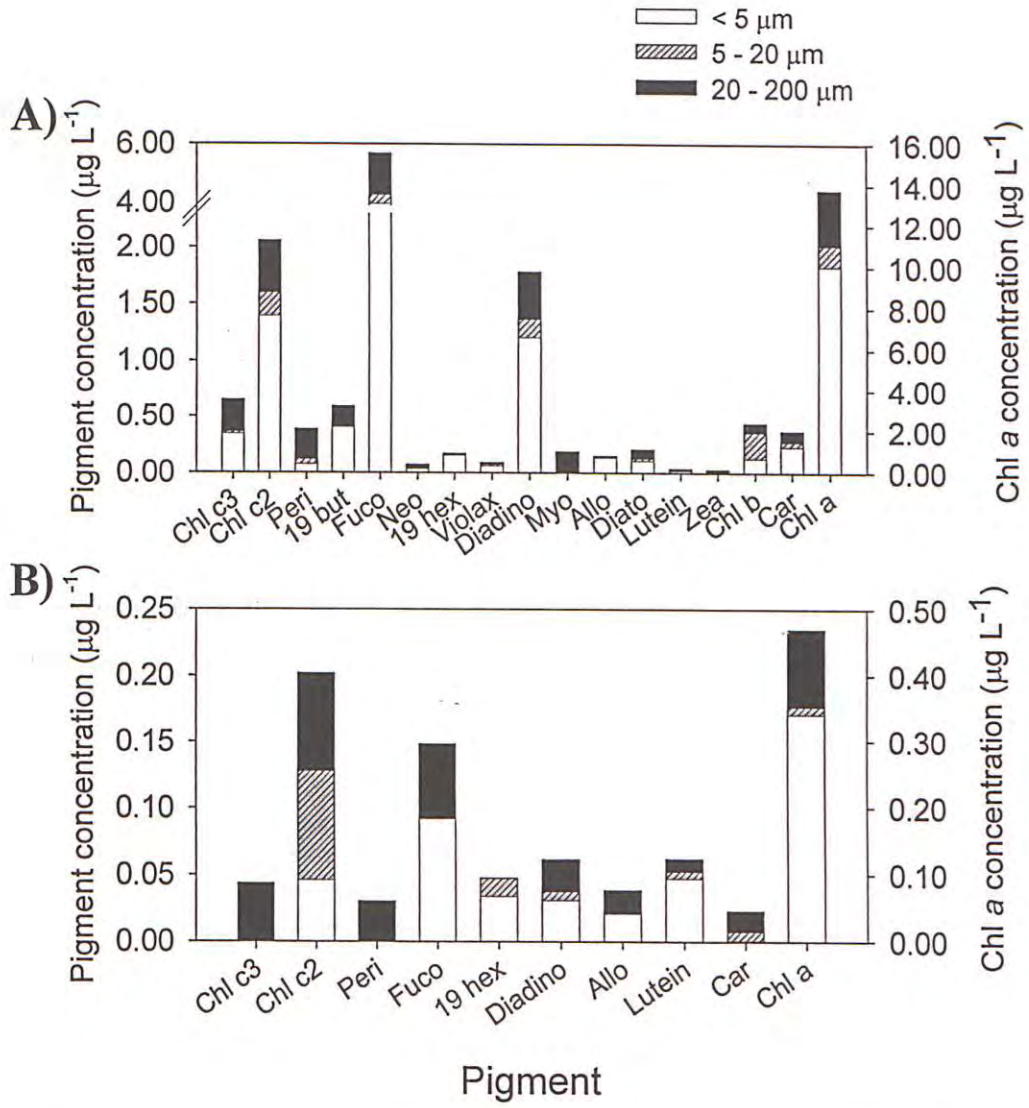


Figure 3.6. Mean concentration of < 5 µm, 5 – 20 µm and 20 – 200 µm phytoplankton pigments in surface seawater collected in TH (A) and MB (B) for dilution experiments in May 07. Refer to table A.1 in Appendix for pigments abbreviations interpretations.

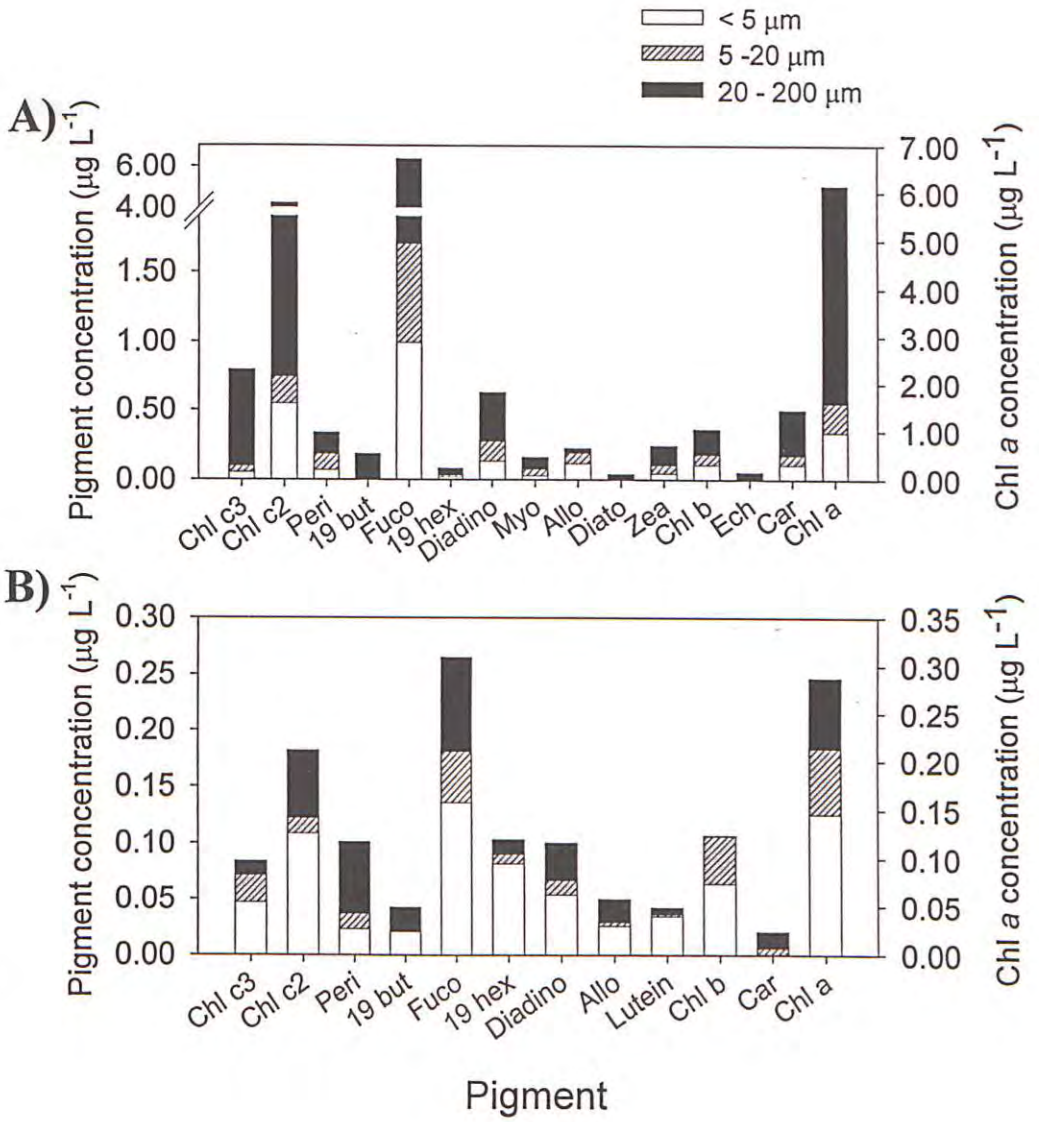


Figure 3.7. Mean concentration of < 5 µm, 5 – 20 µm and 20 – 200 µm phytoplankton pigments in surface seawater collected in TH (A) and MB (B) for dilution experiments in August 07. Refer to table A.1 in Appendix for pigments abbreviation interpretations.

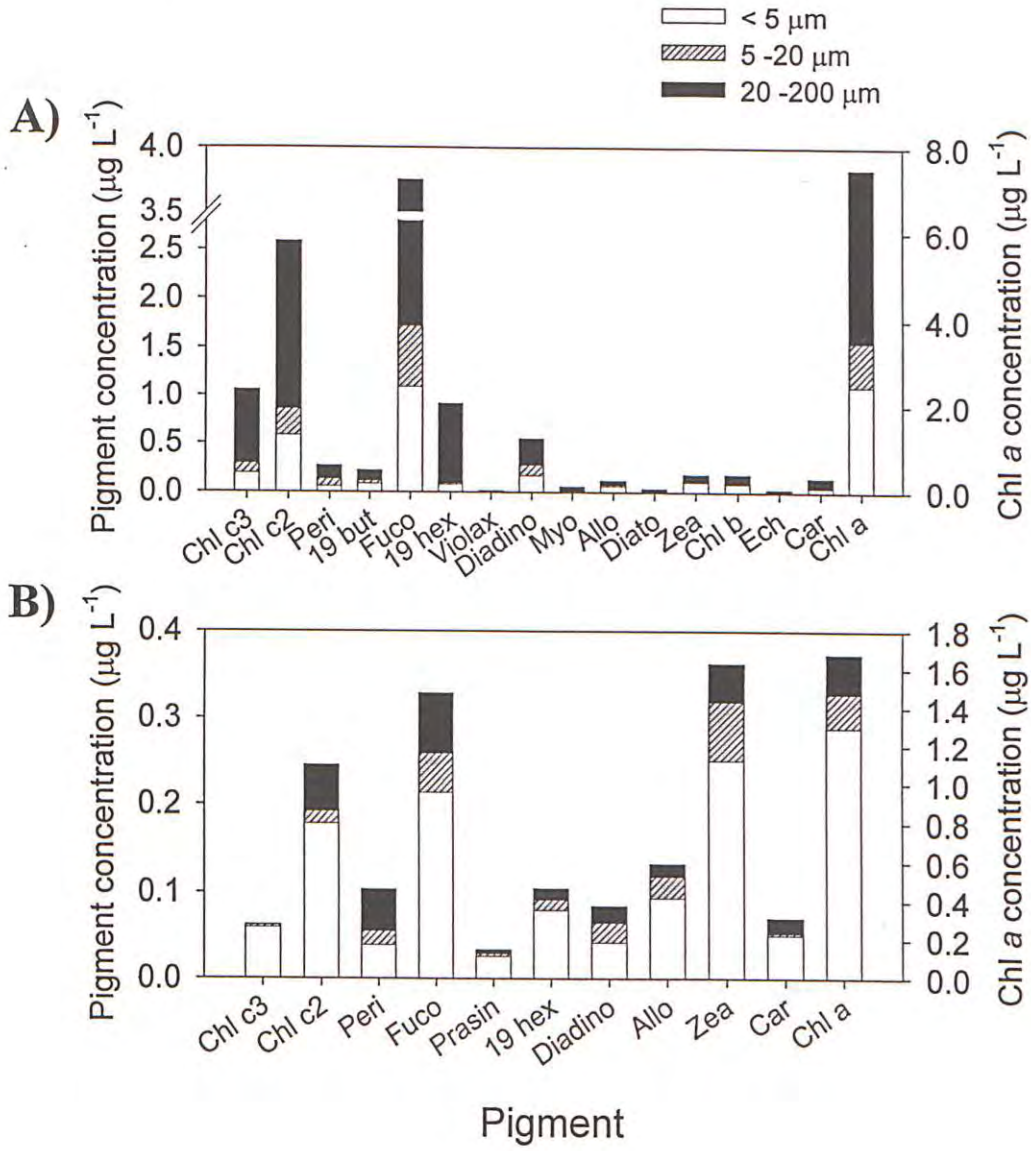


Figure 3.8. Mean concentration of < 5 μm, 5 – 20 μm and 20 – 200 μm phytoplankton pigments in surface seawater collected in TH (A) and MB (B) for dilution experiments in September 07. Refer to table A.1 in Appendix for pigments abbreviation interpretations.

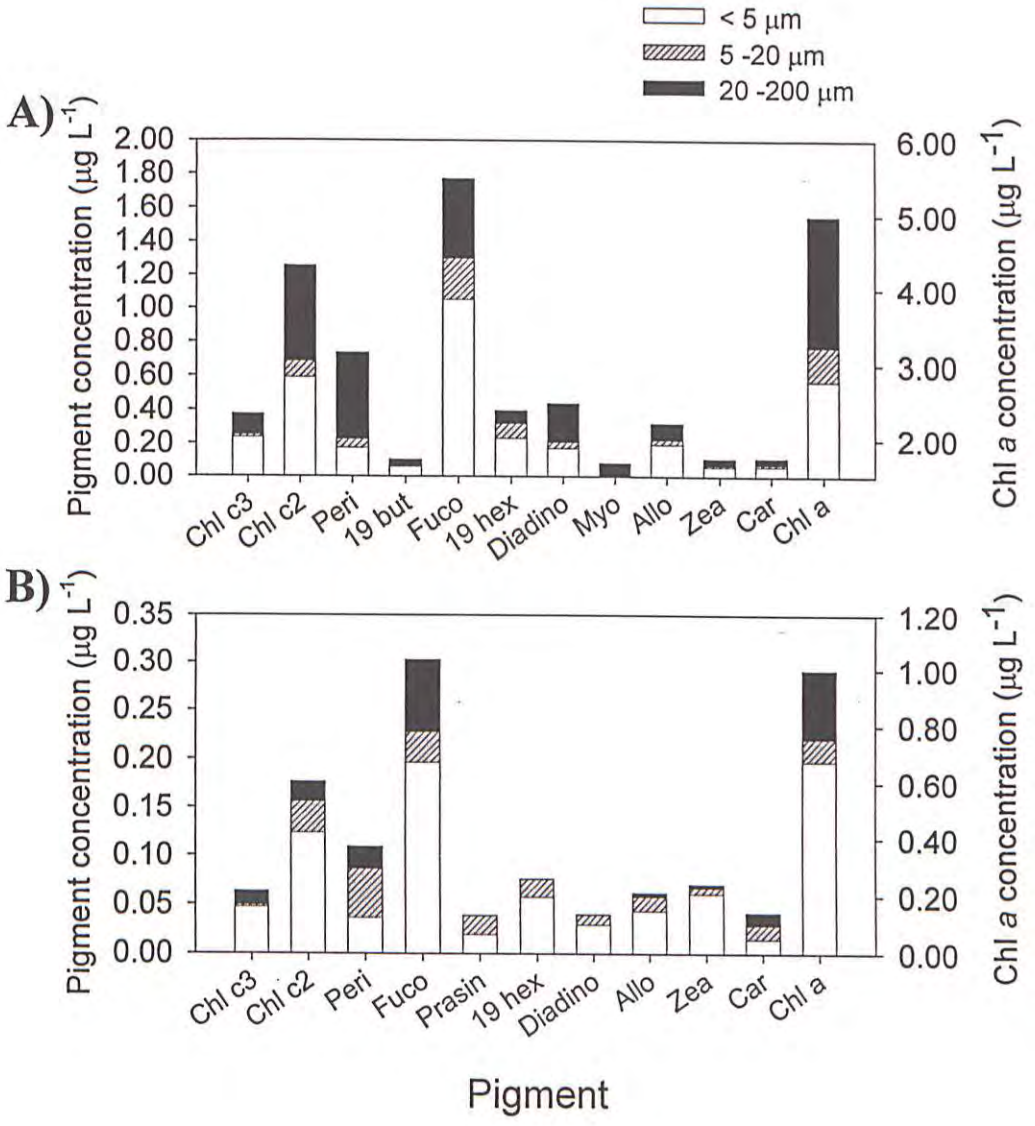


Figure 3.9. Mean concentration of < 5 µm, 5 – 20 µm and 20 – 200 µm phytoplankton pigments in surface seawater collected in TH (A) and MB (B) for dilution experiments in November 07. Refer to table A.1 in Appendix for pigments abbreviation interpretations.

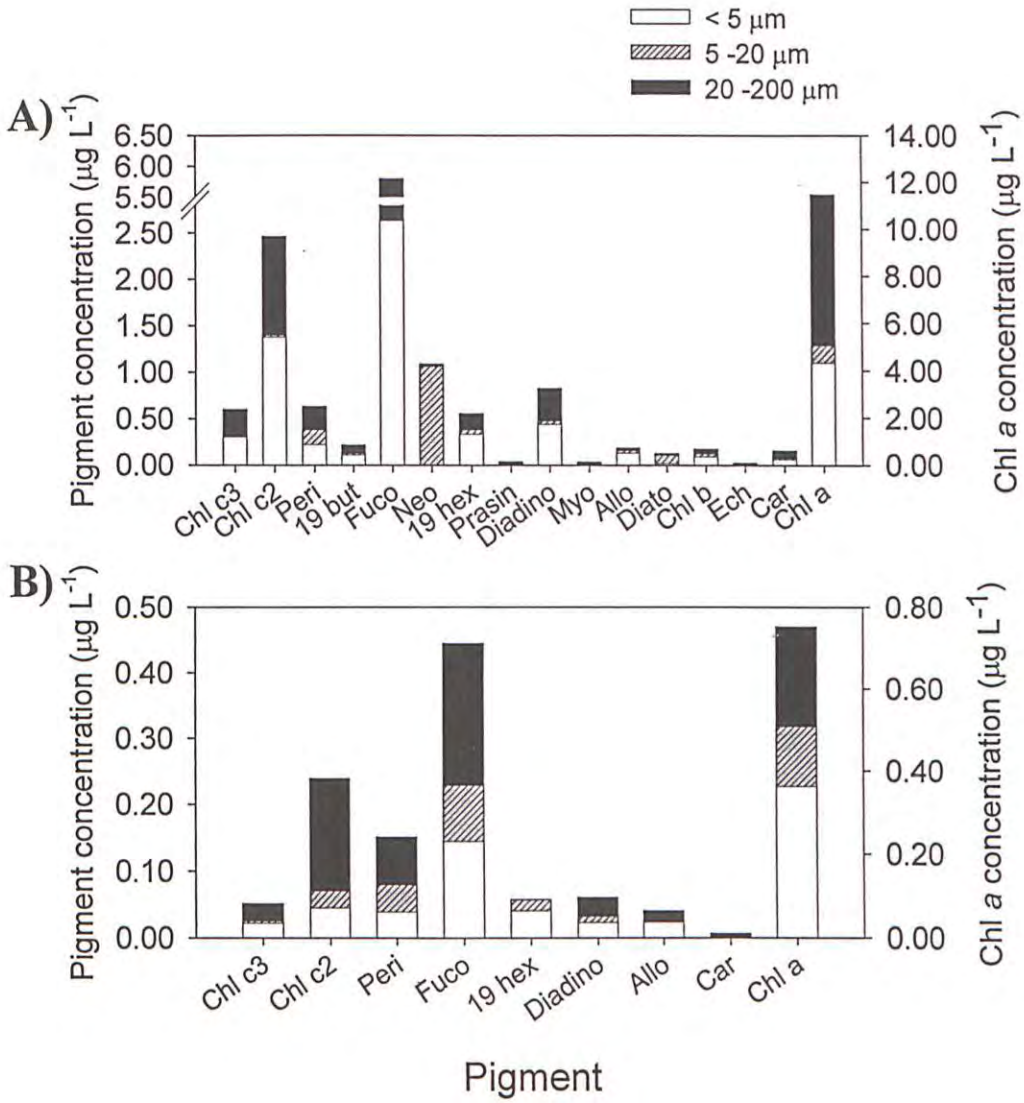


Figure 3.10. Mean concentration of < 5 µm, 5 – 20 µm and 20 – 200 µm phytoplankton pigments in surface seawater collected in TH (A) and MB (B) for dilution experiments in January 08. Refer to table A.1 in Appendix for pigments abbreviation interpretations.

3.2.2. Microscopic cell counts

TH had higher cell counts for all diatoms, dinoflagellates, microzooplankton and other phytoplankton excluding diatoms and dinoflagellates (Others) than MB (Figures 3.11). Densities of dinoflagellates were low in both sites compared to that of other phytoplankton groups, especially in TH where the dinoflagellate density magnitudes can be 3 orders lower than than of diatoms or other phytoplankton. Dinoflagellate densities were mostlly comparable to that of other microzooplankton in MB, but were usually at least several folds higher in TH. Diatoms had the highest density of all groups in most TH samples, and their densities can reach to very high levels ($> 10^5$ numbers ml^{-1}). Microzooplankton densities on the other hand never exceeded 100 numbers ml^{-1}. The group "Others" included all other phytoplankton except dinoflagellates and diatoms, and also unidentified phytoplankton due to limitation in taxonomic expertise. Their densities can be comparable to that of diatoms in TH, and were usually the highest in MB.

Common identified dinoflagellates in both sites included *Prorocentrum, Heterocapsa, Karenia, Gyrodinium,* and *Scrippsiella*, of which *Gyrodinium* is known to be heterotrophic (Figures 3.12 – 3.17). Other examples of heterotrophic dinoflagellates that can be found in both sites included *Peridinium* and *Dinophysis*. A high variety of diatoms were found in both sites. TH usually had a high proportion of small chain forming centric diatoms, which were major contributors to the high chlorophyll *a* concentrations in March, May and January. This was surprising since blooms are generally considered to be associated with larger celled phytoplankton (e.g. Strom et al. 2001, Henjes et al. 2007). Examples of commonly identified diatoms found in both sites were *Pseudo-nitzschia, Chaetoceros, Leptocylindrus,* and *Skeletonema* (Figures 3.12 – 3.17), which save from *Pseudo-nitzschia*, were large

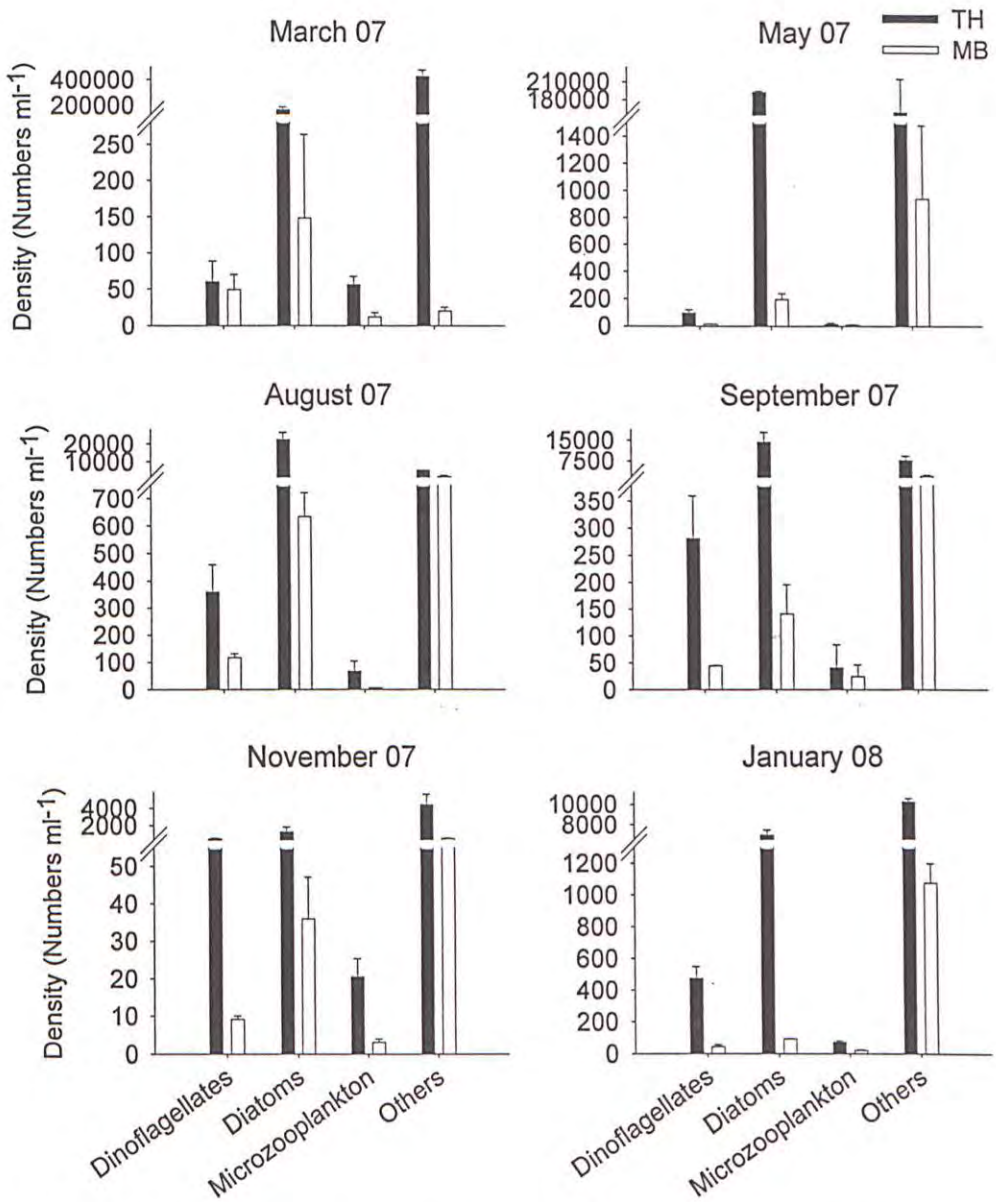


Figure 3.11. Mean density (± standard deviation) of dinoflagellates, diatoms, microzooplankton and others (all phytoplankton except dinoflagellates and diatoms) in Lugol preserved surface seawater samples collected in TH and MB for dilution experiments.

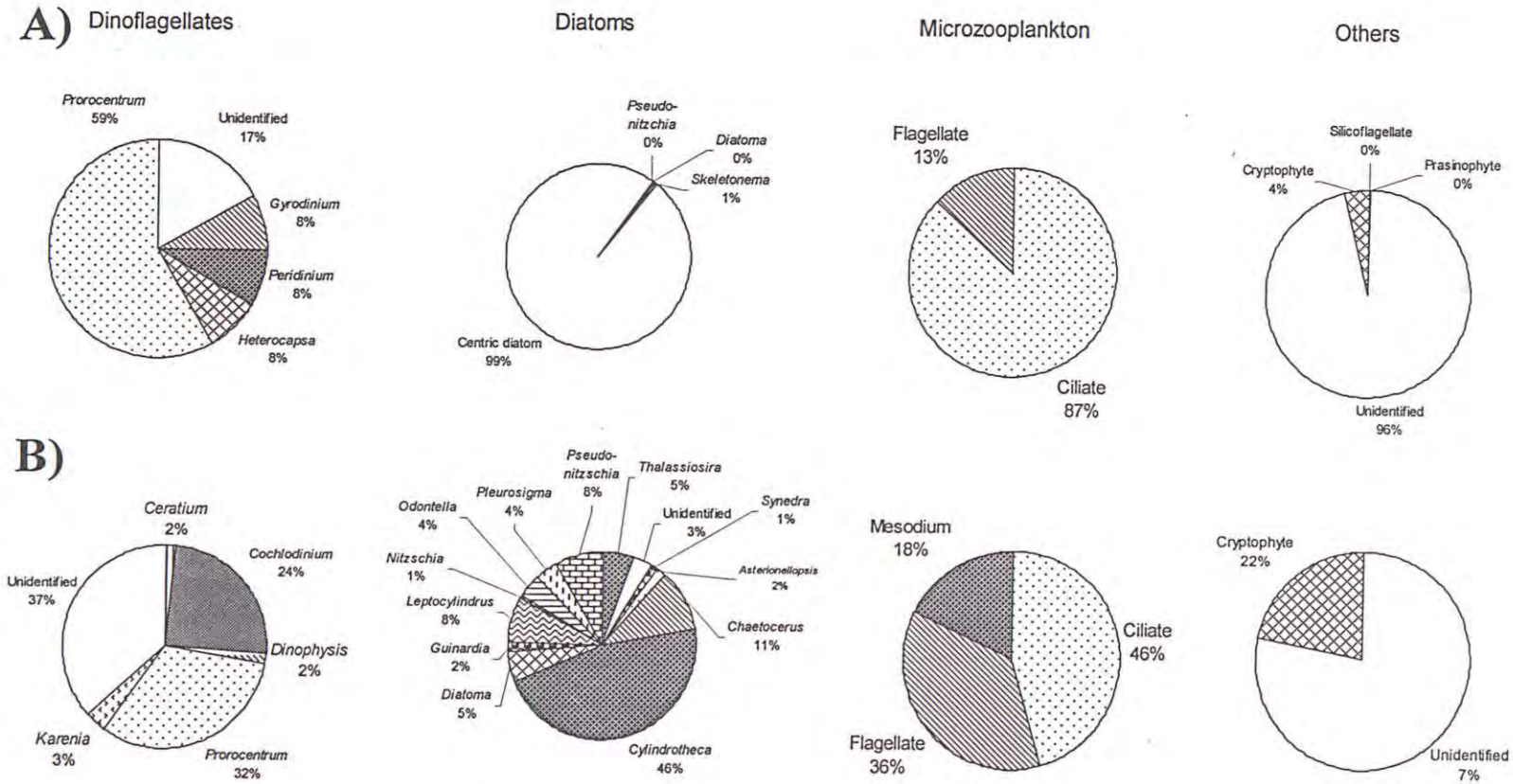


Figure 3.12. Mean percentage of groups or genera of dinoflagellates, diatoms, microzooplankton and others (all phytoplankton except dinoflagellates and diatoms) in Lugol preserved surface seawater samples collected in TH (A) and MB (B) for dilution experiments in March 07.

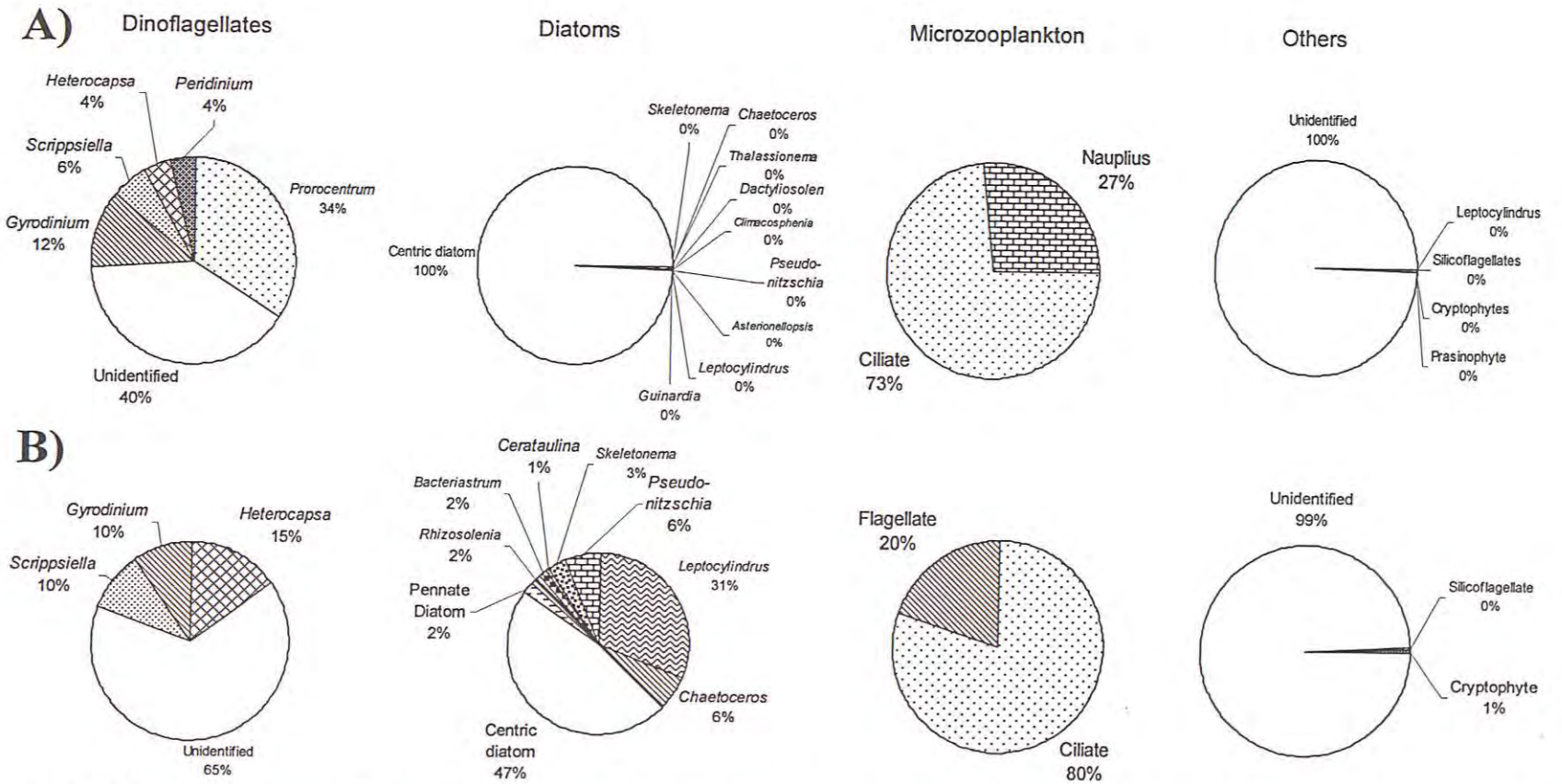


Figure 3.13. Mean percentage of groups or genera of dinoflagellates, diatoms, microzooplankton and others (all phytoplankton except dinoflagellates and diatoms) in Lugol preserved surface seawater samples collected in TH (A) and MB (B) for dilution experiments in May 07.

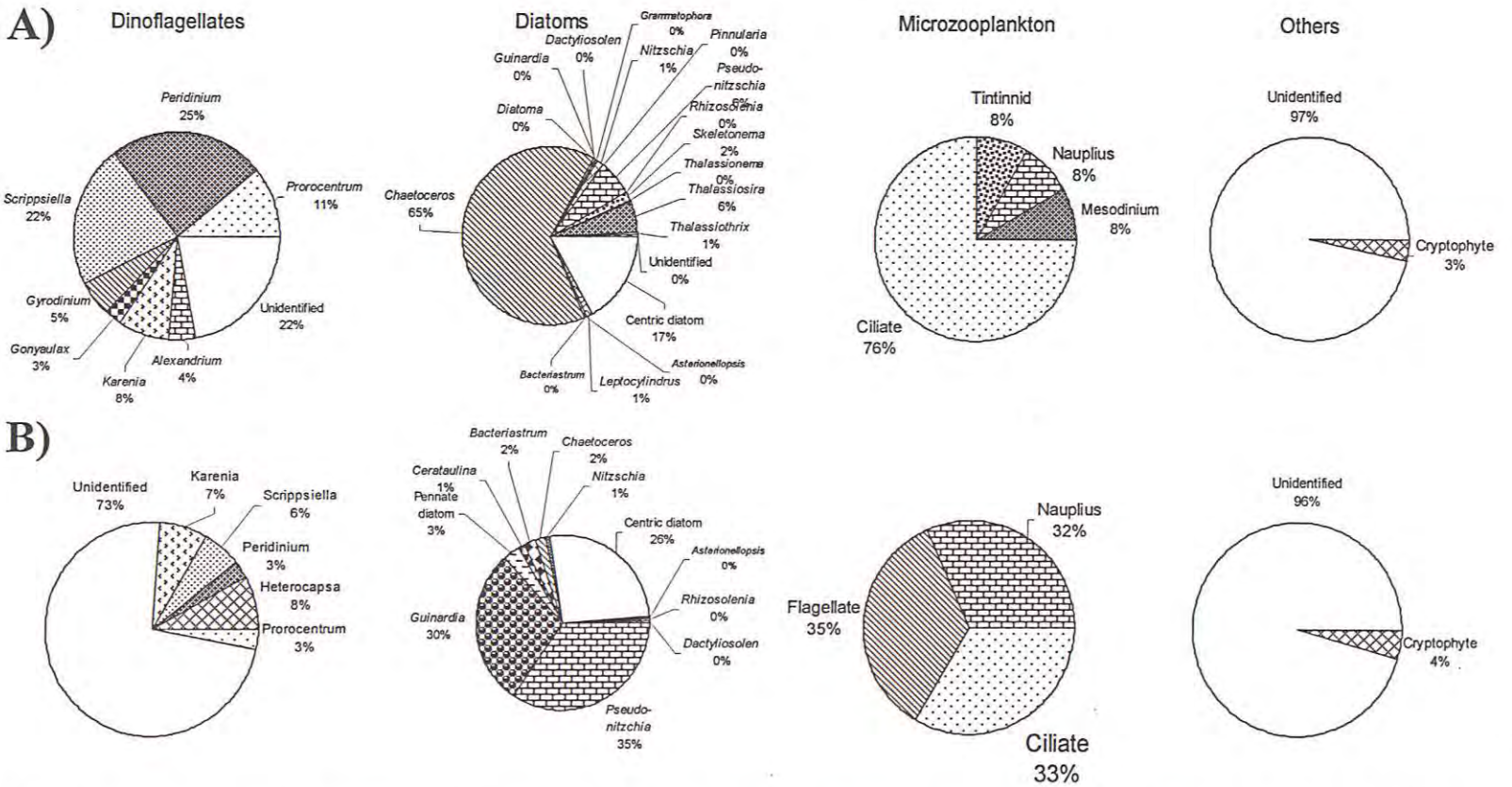


Figure 3.14. Mean percentage of groups or genera of dinoflagellates, diatoms, microzooplankton and others (all phytoplankton except dinoflagellates and diatoms) in Lugol preserved surface seawater samples collected in TH (A) and MB (B) for dilution experiments in August 07.

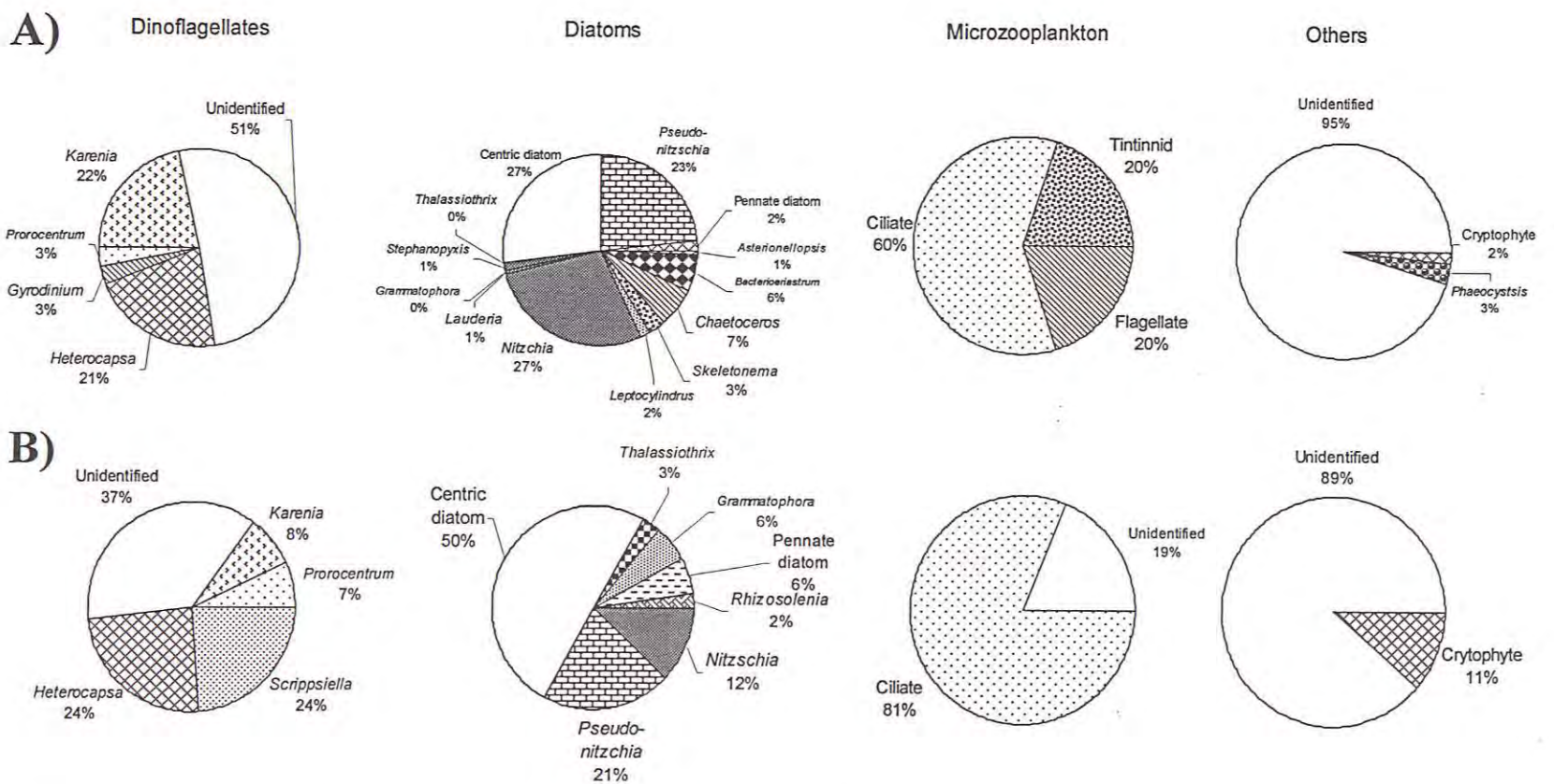


Figure 3.15. Mean percentage of groups or genera of dinoflagellates, diatoms, microzooplankton and others (all phytoplankton except dinoflagellates and diatoms) in Lugol preserved surface seawater samples collected in TH (A) and MB (B) for dilution experiments in September 07.

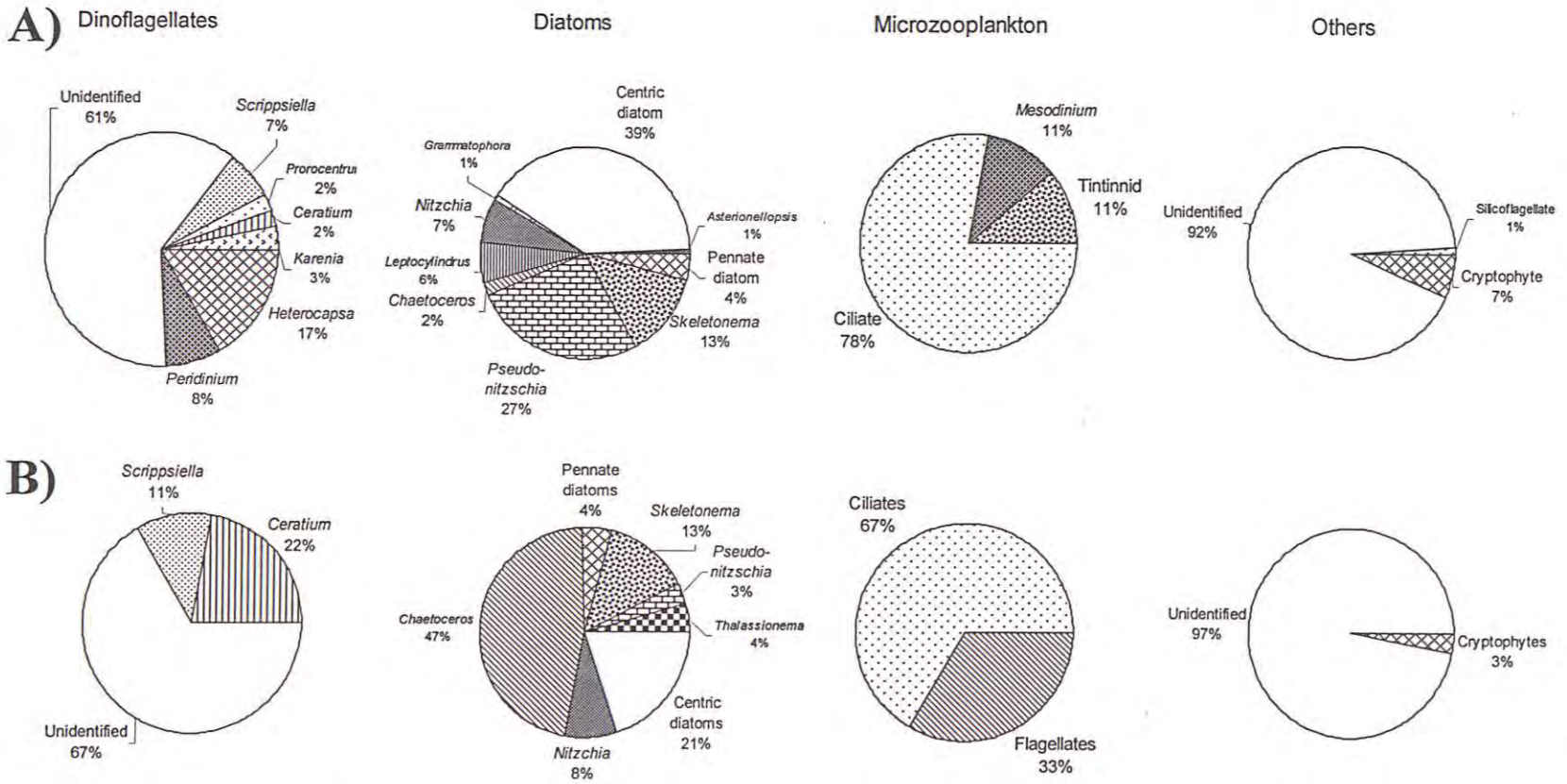


Figure 3.16. Mean percentage of groups or genera of dinoflagellates, diatoms, microzooplankton and others (all phytoplankton except dinoflagellates and diatoms) in Lugol preserved surface seawater samples collected in TH (A) and MB (B) for dilution experiments in November 07.

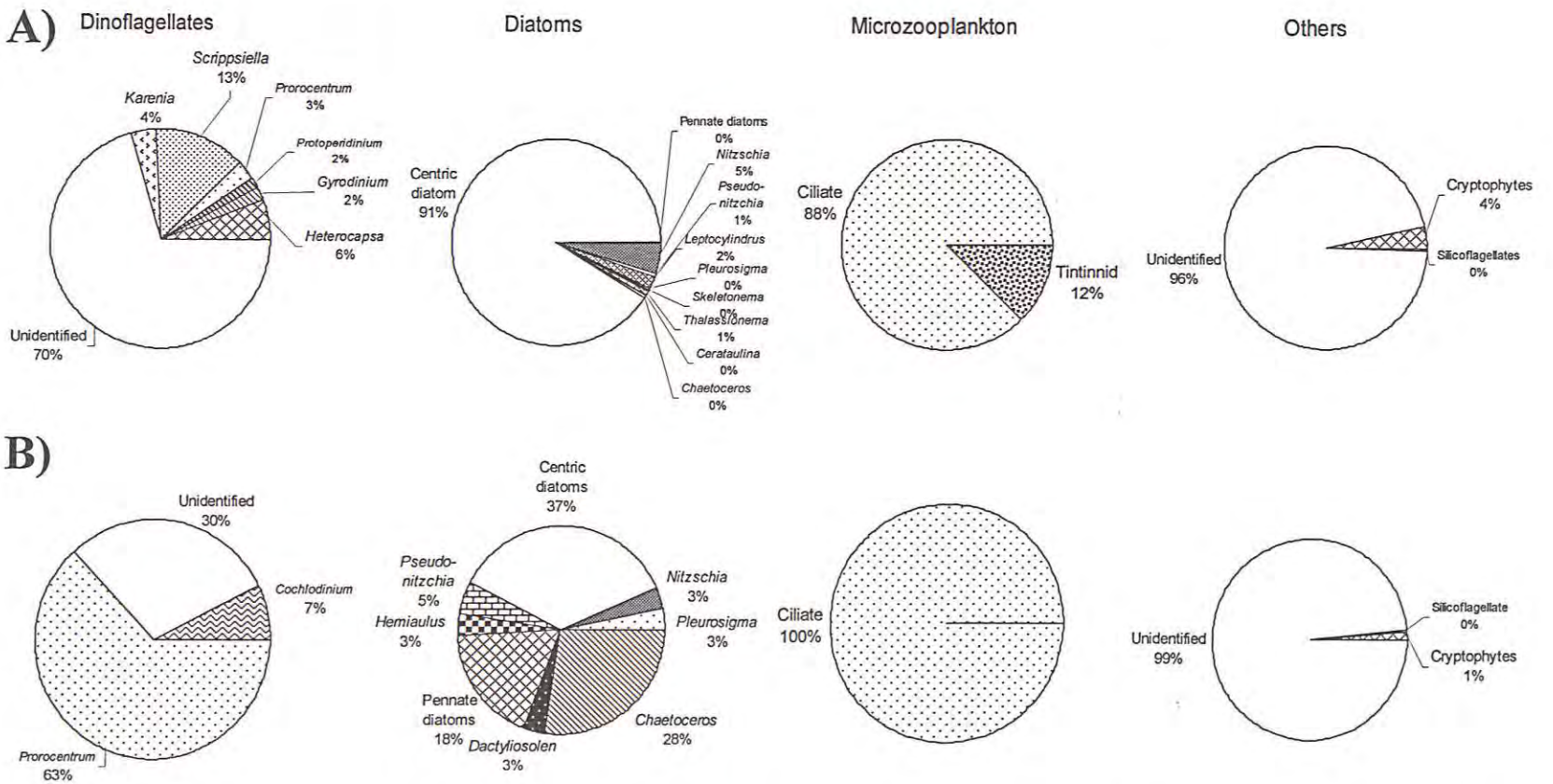


Figure 3.17. Mean percentage of groups or genera of dinoflagellates, diatoms, microzooplankton and others (all phytoplankton except dinoflagellates and diatoms) in Lugol preserved surface seawater samples collected in TH (A) and MB (B) for dilution experiments in January 08.

chain forming centric diatoms. Ciliates, including tintinnids and oligotrichs, were the most commonly found microzooplankton in both sites (Figures 3.12 – 3.17). Cryptophytes and silicoflagellates were commonly found in the samples, but were grouped into "Others" due to their low densities. As mentioned, due to limitation in taxonomic skills, a high proportion of "Others" in both sites were small unidentified cells (Figures 3.12 – 3.17).

3.3. Dilution experiments results

3.3.1. Linear regression analysis results

Linear regression analyses were made only when there were more than three data points available for the pigment marker ($n > 3$). Uninterpretable analyses due to insignificant ($p > 0.05$) and positive slopes were common. The number of points available for analysis (n) was at times smaller in MB than in TH since HPLC could not detect the low pigment concentrations in MB (Tables A.2 – A.7 in Appendix). It should be noted that attempts to calculate 20 – 200 μm and 5 – 20 μm g were made through calculations (data not shown), but few significant slopes were yielded. In addition, although pigment concentrations of different size fractions were obtained from identical replicates, they nevertheless came from different incubations, so it was deemed inappropriate to obtain 20 – 200 μm and 5 – 20 μm g through calculations.

Data from linear regression analyses needed to be revised in the cases of uninterpretable results (See section 2.2.3), which were common in cases regarding the < 5 μm size fraction and pigments with low concentrations. Of all thirty-six dilution experiments performed (Three size fractions for each site in six experimental months), only six experiments gave no g on all pigment markers. Of

these six experiments, two were for the < 20 μm size fraction, and the remaining were for < 5 μm. < 5 μm dilution experiments frequently gave less estimatable *g* than the other two larger size fractions. There was no pigment marker that gave estimatable *g* in all dilution experiments, but fucoxanthin and chlorophyll *a* yielded more estimatable *g* than other pigment markers. This supports Dolan and McKeon's (2005) claim that the dilution method is insensitive towards low grazing rates when slight changes in pigment concentrations needs to be detected to generate a significant *g*.

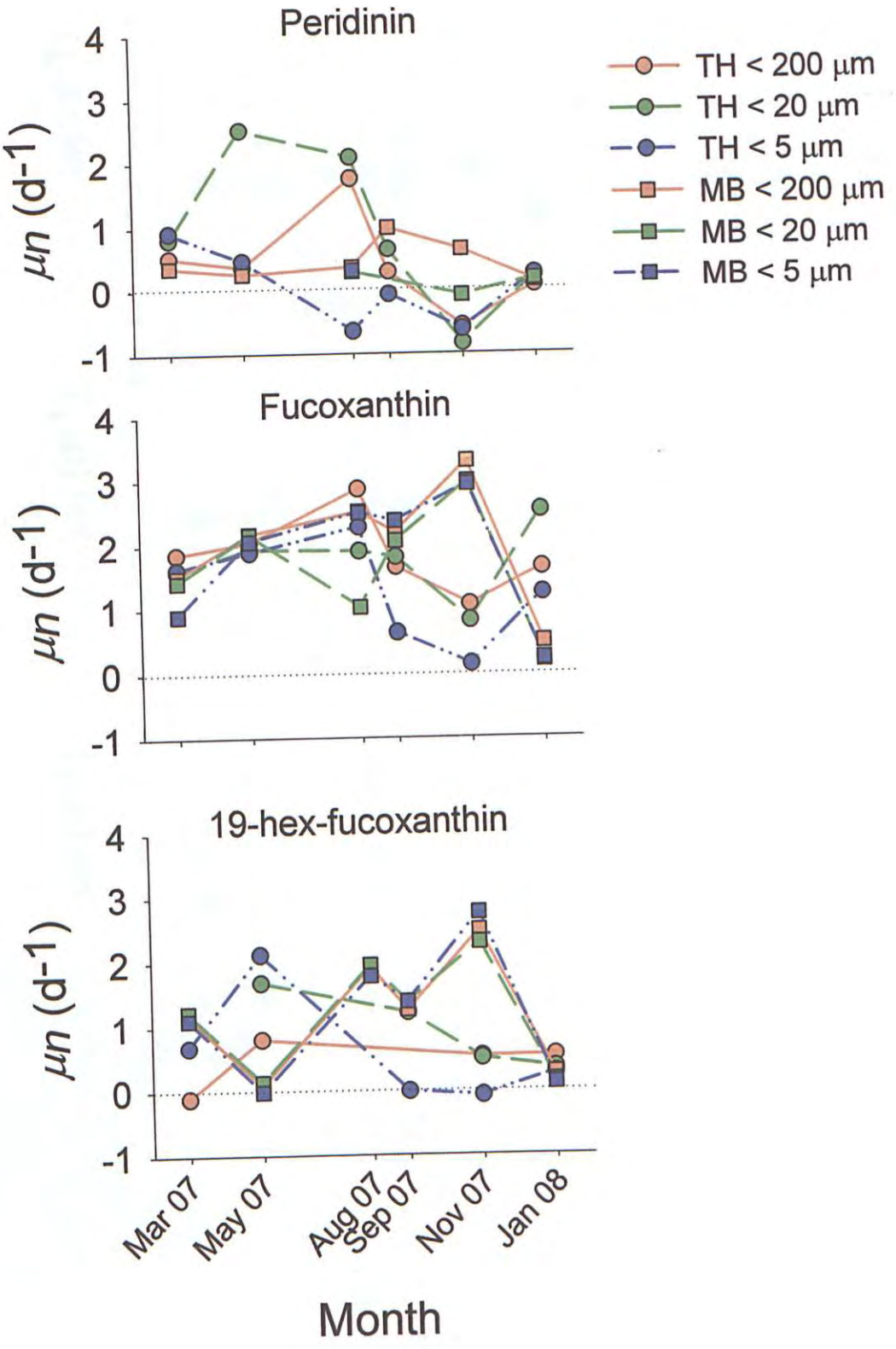
The ranges of the percentage of standing stock (SS) grazed for various pigment markers in TH were 24.5 – 92.0%, 30.7 – 97.6% and 41.4 – 89.6% for < 200 μm, < 20 μm and < 5 μm respectively. In MB, the ranges were 20.4 – 77.2%, 38.4 – 80.2% and 47.9 – 80.6% for < 200 μm, < 20 μm and < 5 μm respectively. The averages of SS grazed for all pigment markers in each size fraction were similar, and the overall average for all pigment markers of all size fractions for TH and MB were 62.2% and 59.7% respectively (Tables A.8 – A.13 in Appendix).

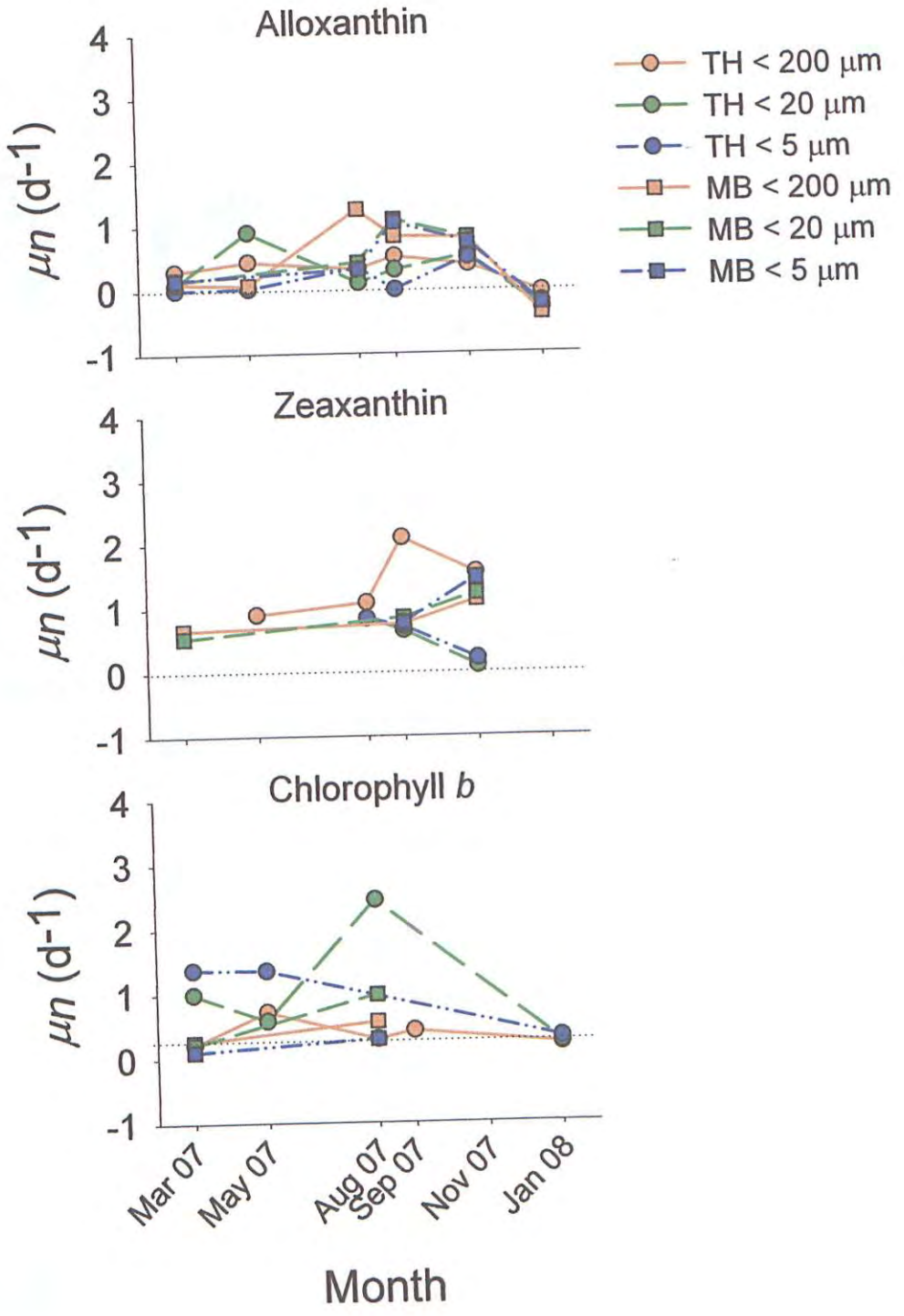
Despite the slightly higher SS grazed in November in MB (~60% in all size fractions) than in TH (~50% in all size fractions), the percentage of production (Production) grazed was much higher in TH (> 200%) than in MB (< 100%), due to the high μ_0 in MB (> 2 d^{-1} for most pigments) at that time (Tables A.8 – A.13 in Appendix). The Production grazed in TH in November was in fact generally the highest for that site, corresponding to the lowest chlorophyll *a* concentrations of the site throughout the whole study period (Figure 3.3A). The overall highest Production grazed in MB was in September, which unlike in TH, corresponded to the highest chlorophyll *a* concentration of that site throughout the whole study period (Figure 3.3B), which might have been the start of the grazers' response towards the high

productivity. Production grazed was always higher in TH. The ranges for various pigment markers were 90.4 – 3508.6%, 98.2 – 797.4% and 90.1 – 3573.7% for < 200 μm, < 20 μm and < 5 μm respectively. In MB, the ranges were 56.6 – 283.0%, 49.7 – 167.3% and 66.4 – 149.9% for < 200 μm, < 20 μm and < 5 μm respectively. For both sites, maximum values were all from high Production grazed on alloxanthin.

3.3.2. Estimated pigment specific phytoplankton growth rates and microzooplankton grazing rates

Pigment specific phytoplankton potential growth rates (μ_n) were mostly positive due to nutrient enrichment (Figure 3.18). μ_n were generally comparable between TH and MB and among the three size fractions. In November 07 however, TH had relatively low μ_n for most pigment markers while MB had the highest μ_n for most pigment markers. Alloxanthin was the only pigment marker exempt from this phenomenon in that it had the least variable μ_n among all seven pigment markers (TH: -0.29 – 0.90 d^{-1} and MB: -0.38 – 1.25 d^{-1}). μ_n of various pigment markers except alloxanthin at both sites and among the three size fractions were comparable for each experimental month. μ_n of alloxanthin were also similar between the two sites and among the three size fractions, but the values were slightly lower compared to the other pigment markers, especially in TH, where the maximum value was ~3X lower than that of other pigment markers. This indicates that cryptophytes had lower growth rates than other groups in this study. But these growth rates obtained does not seem to be low considering the growth rate of *Cryptomonas* sp. in an experiment under high light and N enrichment was ~ 1 d^{-1} (Sciandra et al. 2000).


Peridinin
Fucoxanthin
19-hex-fucoxanthin
μn (d-1)
4
3
2
1
0
-1
TH < 200 μm
TH < 20 μm
TH < 5 μm
MB < 200 μm
MB < 20 μm
MB < 5 μm
Mar 07
May 07
Aug 07
Sep 07
Nov 07
Jan 08
Month


Alloxanthin
Zeaxanthin
Chlorophyll b
μn (d-1)
4
3
2
1
0
-1
TH < 200 μm
TH < 20 μm
TH < 5 μm
MB < 200 μm
MB < 20 μm
MB < 5 μm
Mar 07
May 07
Aug 07
Sep 07
Nov 07
Jan 08
Month

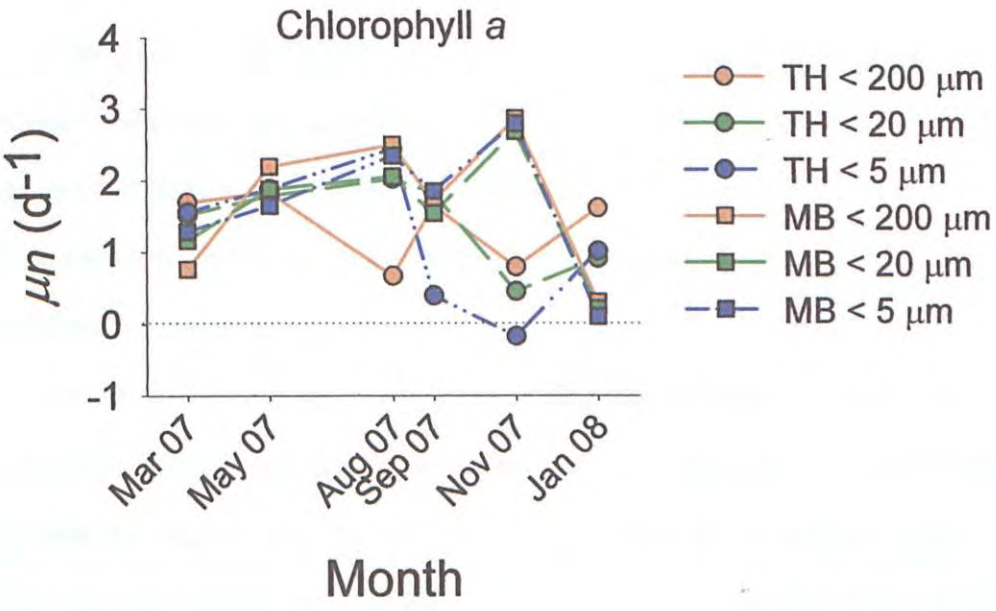


Figure 3.18. Temporal variations in the estimated pigment specific phytoplankton potential growth rates (μ_n) of peridinin, fucoxanthin, 19-hex-fucoxanthin, alloxanthin, zeaxanthin, chlorophyll *b* and chlorophyll *a* of < 200 μm, < 20 μm and < 5 μm phytoplankton for the dilution experiments in TH and MB during the study period of March 07 – January 08. Missing points are due to unavailable data.

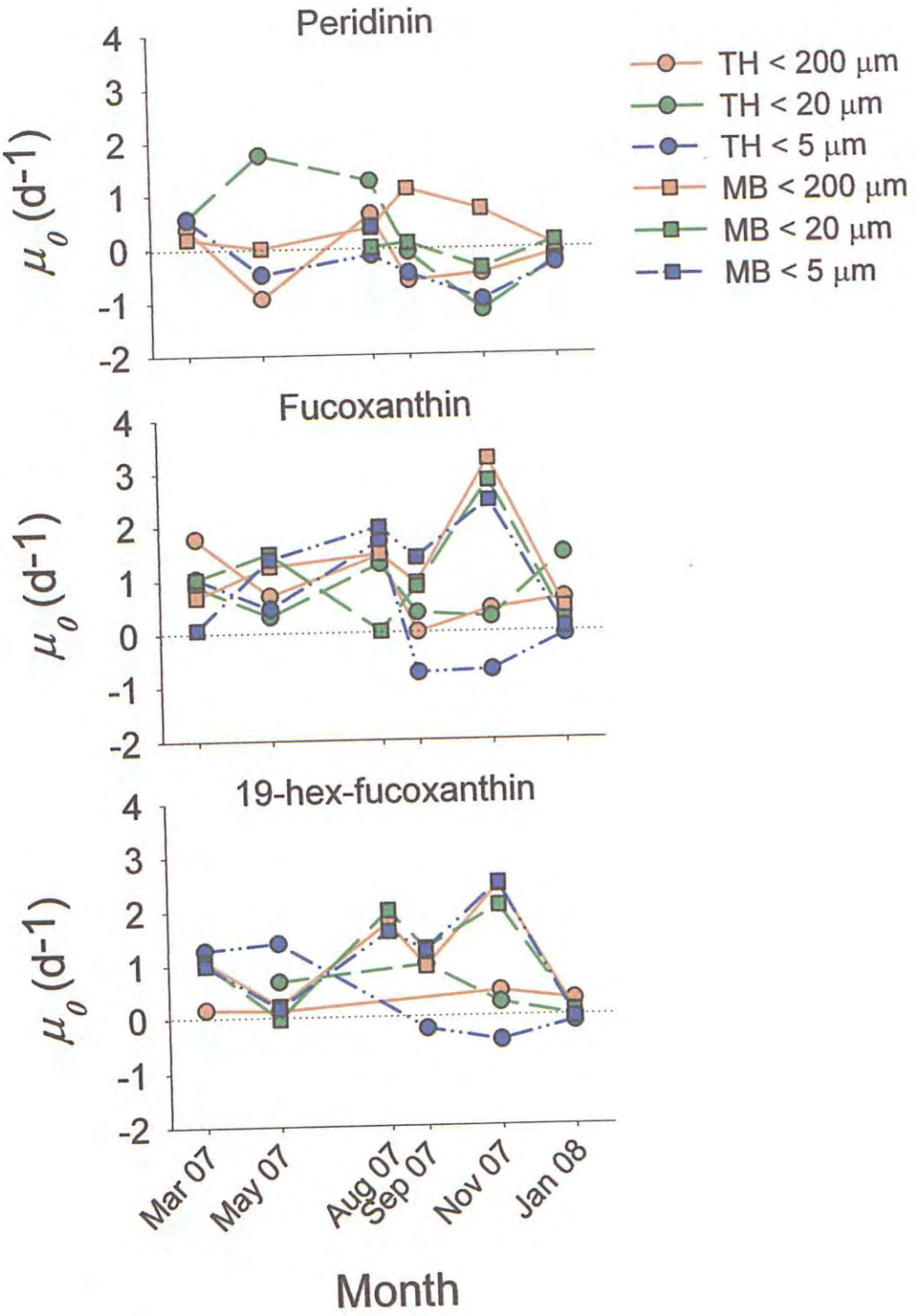
The estimated pigment specific phytoplankton growth rate in ambient nutrients (μ_0) of all pigment markers were usually slightly lower than μ_n, but showed similar seasonal trends as μ_n (Figure 3.19). This indicates that although ambient nutrients were not sufficient to provide maximum growth, they were not significantly limiting. There were a few cases where μ_0 was slightly higher than μ_n, most probably due to variations in estimation.

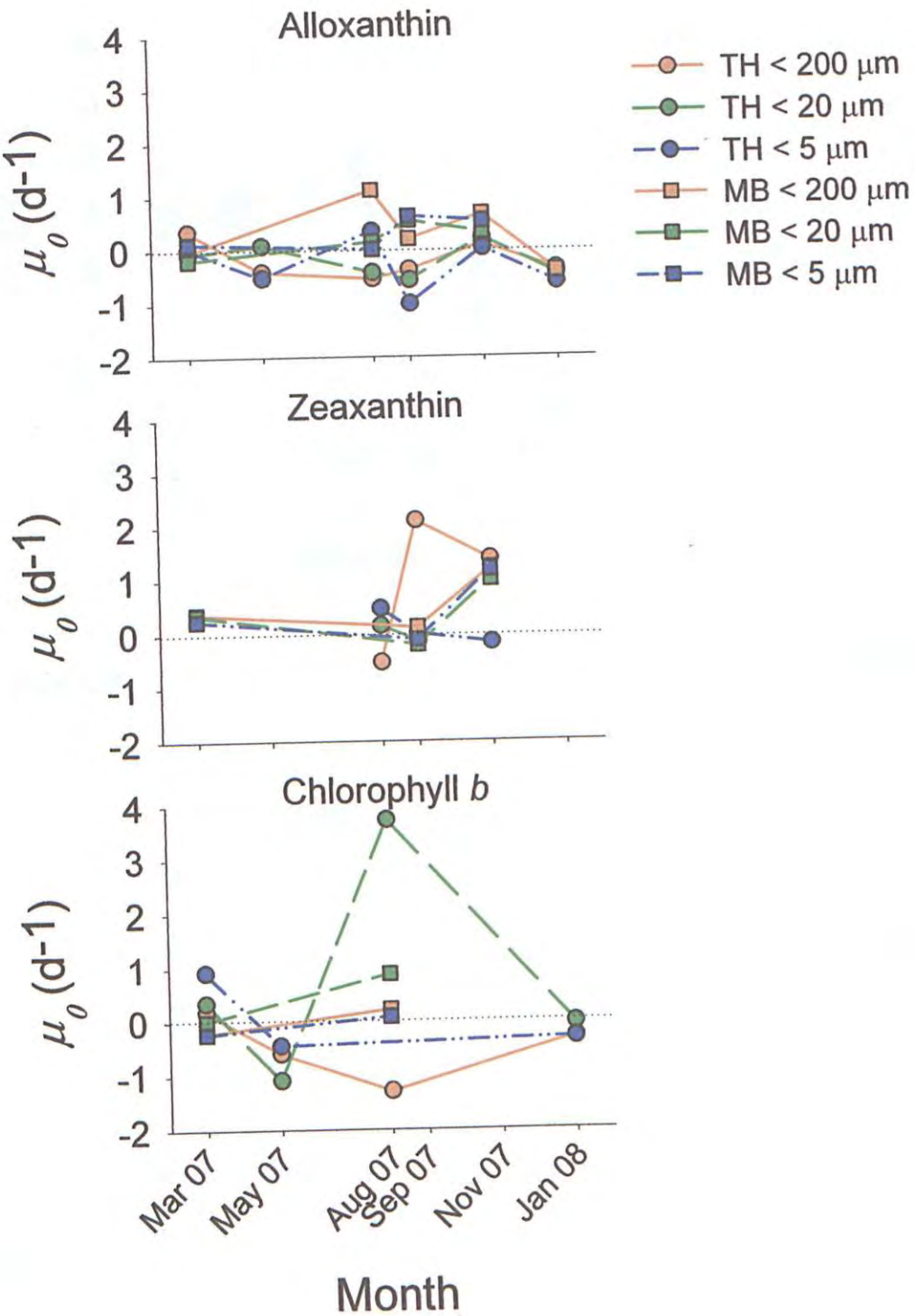
Similar to the phytoplankton growth rates, the estimated pigment specific microzooplankton grazing rate (g) were comparable between both sites and among the three size fractions (Figure 3.20). Variations, such as higher rates in August and lower rates in January, were similar between g and μ, except g in MB were not particularly high in November considering the high μ in the same month and site.

3.3.3. Ratio of microzooplankton grazing to the phytoplankton growth rate in ambient nutrients

The ratio of the estimated pigment specific microzooplankton grazing rate to the estimated pigment specific phytoplankton growth rate in ambient nutrients (g/μ_0) shows the control of microzooplankton grazing on phytoplankton growth. > 1 value indicates that the grazing rate is higher than the growth rate so that the grazing control is high. < 1 value indicates that the grazing rate is lower than the growth rate, so that the control is low. At 1, it indicates that the growth rate is balanced by the grazing rate.

Although μ_0 and g were comparable between TH and MB, g/μ_0 in TH were usually higher and > 1 (Figure 3.21). This shows that microzooplankton grazing control was higher in TH. Extremely high values (> 40) were also found in TH only for alloxanthin (45.84 and 44.57) and fucoxanthin (44.98), but these high values were due to low growth rates instead of high grazing rates. High g, such as on


Peridinin
Fucoxanthin
19-hex-fucoxanthin
μ_0 (d-1)
4
3
2
1
0
-1
-2
TH < 200 µm
TH < 20 µm
TH < 5 µm
MB < 200 µm
MB < 20 µm
MB < 5 µm
Mar 07
May 07
Aug 07
Sep 07
Nov 07
Jan 08
Month


Alloxanthin
Zeaxanthin
Chlorophyll b
μ_0 (d-1)
4
3
2
1
0
-1
-2
TH < 200 µm
TH < 20 µm
TH < 5 µm
MB < 200 µm
MB < 20 µm
MB < 5 µm
Mar 07
May 07
Aug 07
Sep 07
Nov 07
Jan 08
Month

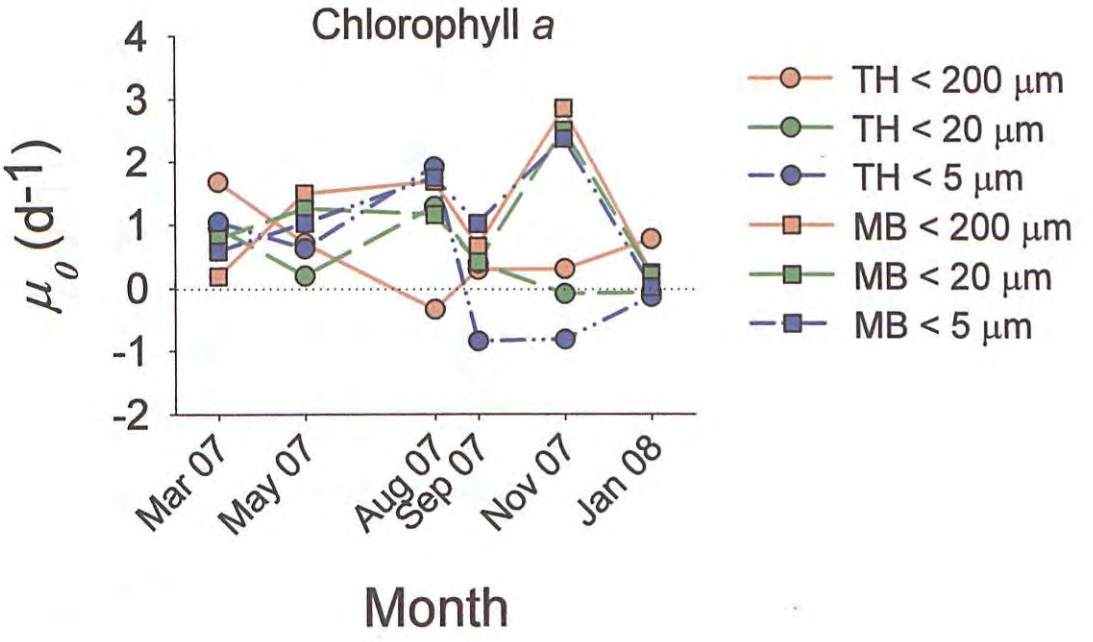
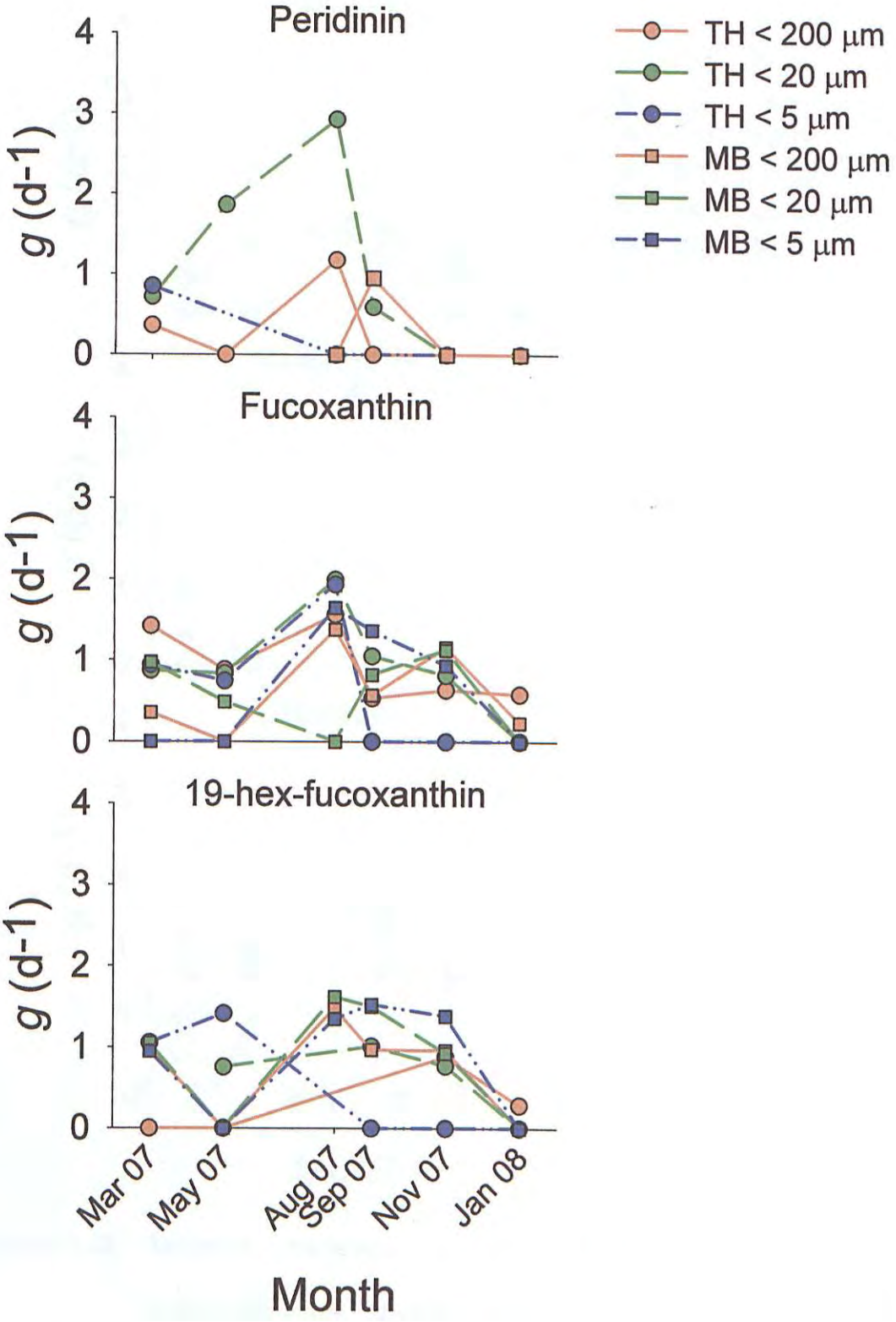


Figure 3.19. Temporal variations in the estimated pigment specific phytoplankton growth rates in ambient nutrients (μ_0) of peridinin, fucoxanthin, 19-hex-fucoxanthin, alloxanthin, zeaxanthin, chlorophyll *b* and chlorophyll *a* of < 200 μm, < 20 μm and < 5 μm phytoplankton for the dilution experiments in TH and MB during the study period of March 07 – January 08. Missing points are due to unavailable data.


Peridinin
Fucoxanthin
19-hex-fucoxanthin
g (d-1)
0
1
2
3
4
TH < 200 µm
TH < 20 µm
TH < 5 µm
MB < 200 µm
MB < 20 µm
MB < 5 µm
Mar 07
May 07
Aug 07
Sep 07
Nov 07
Jan 08
Month

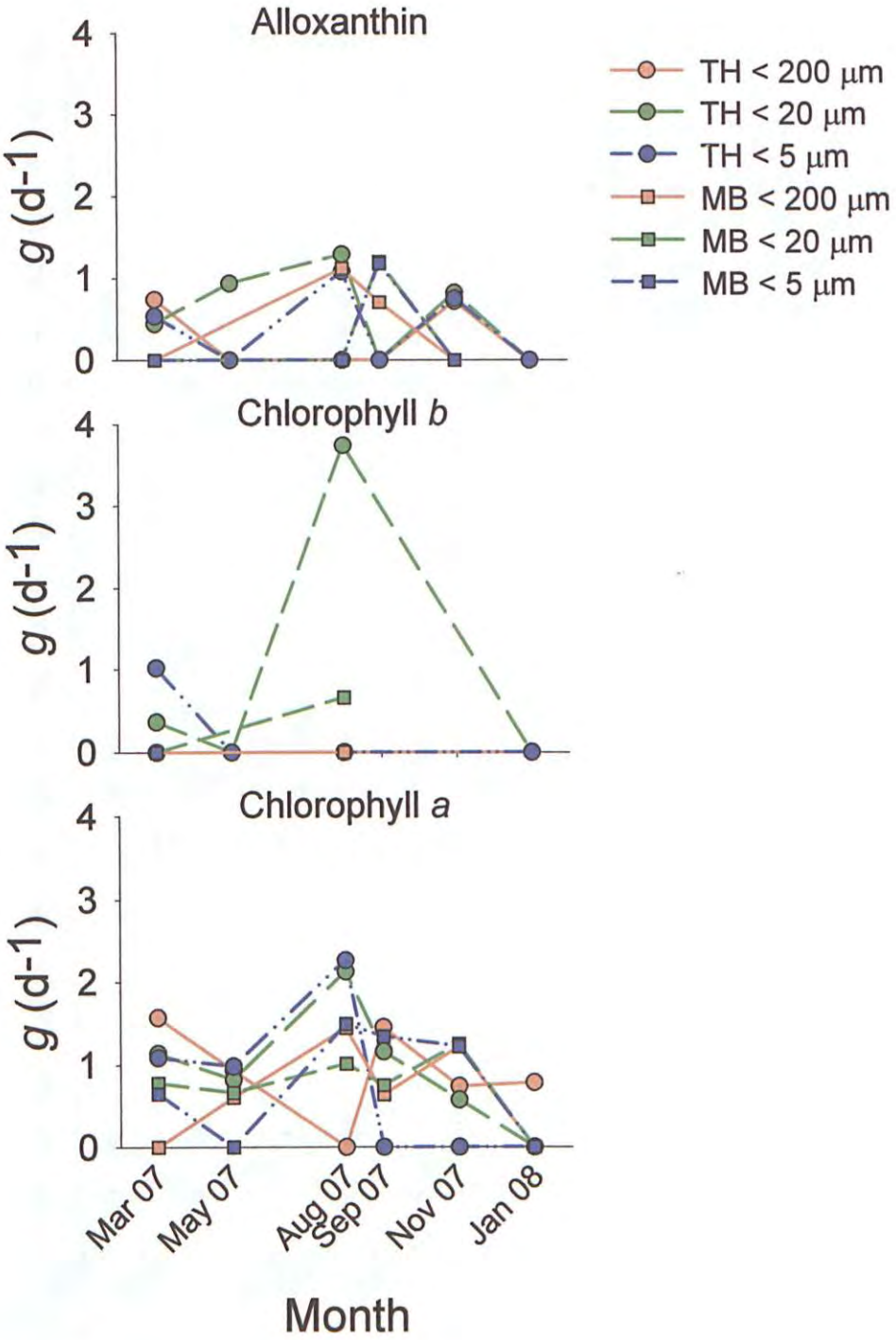
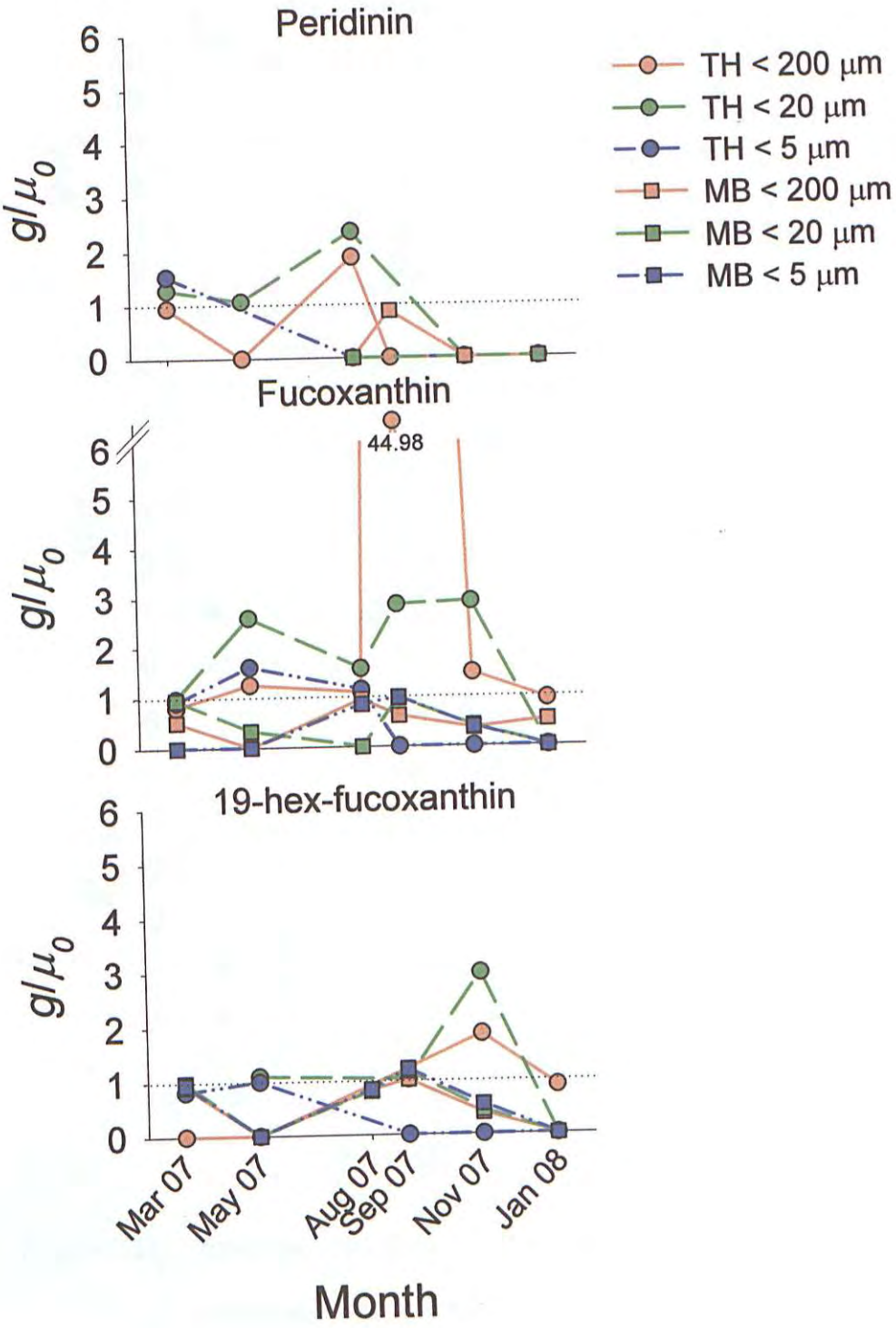


Figure 3.20. Temporal variations in the estimated pigment specific microzooplankton grazing rates (*g*) of peridinin, fucoxanthin, 19-hex-fucoxanthin, alloxanthin, chlorophyll *b* and chlorophyll *a* of < 200 µm, < 20 µm and < 5 µm phytoplankton for the dilution experiments in TH and MB during the study period of March 07 – January 08. Missing points are due to unavailable data.


Peridinin
Fucoxanthin
19-hex-fucoxanthin
g/μ_0
44.98
TH < 200 µm
TH < 20 µm
TH < 5 µm
MB < 200 µm
MB < 20 µm
MB < 5 µm
Mar 07
May 07
Aug 07
Sep 07
Nov 07
Jan 08
Month

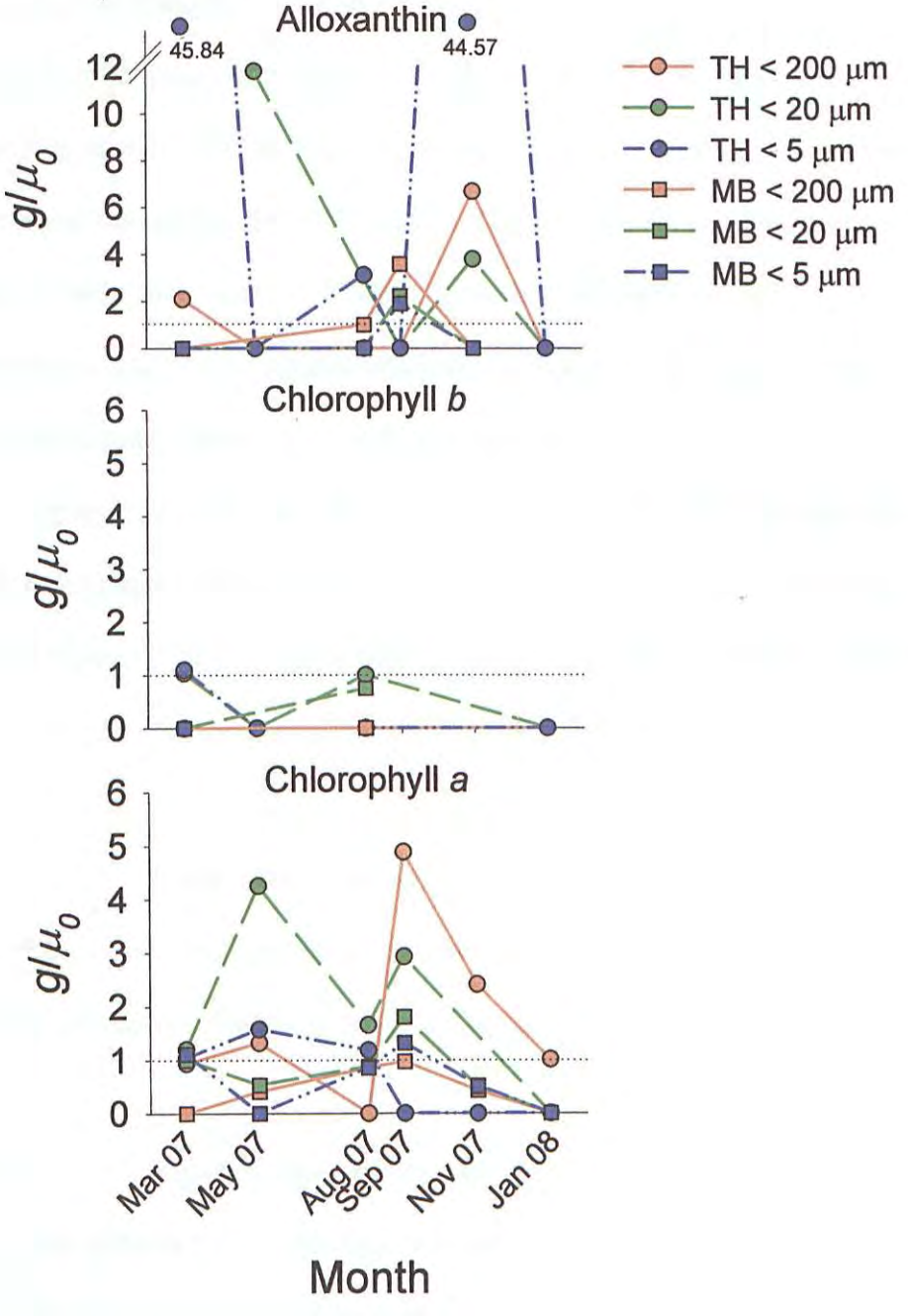


Figure 3.21. Temporal variations in the ratios of estimated pigment specific microzooplankton grazing rates to the estimated pigment specific phytoplankton growth rates in ambient nutrients (g/μ_0) of peridinin, fucoxanthin, 19-hex-fucoxanthin, alloxanthin, chlorophyll *b* and chlorophyll *a* of < 200 μm, < 20 μm and < 5 μm phytoplankton for the dilution experiments in TH and MB during the study period of March 07 – January 08. Missing points are due to unavailable data.

< 20 μm chlorophyll *b* in TH in August 07, were often met with high μ_0 and did not result in high g/μ_0. Although g/μ_0 for the < 200 μm size fraction were often lower than that of the < 20 μm and < 5 μm size fractions, the trend was not obvious and consistent throughout the study period. The g/μ_0 for alloxanthin in all size fractions and in both sites were > 1 when g wasn't assumed to be 0. This shows that alloxanthin was heavily grazed relatively to its low μ_0, and may indicate a preference of local microzooplankton towards cryptophytes.

Figure 3.22 is another expression of the g/μ_0 ratio, and with the regression and equilibrium curve, it shows that g in TH should be higher than μ_0 until μ_0 reaches to higher than ~ 1.5 d^{-1}. While in MB, g is only higher than μ_0 when it is less than ~ 0.5 d^{-1}.

3.4. Correlation analyses

Pearson's correlation analyses were performed to study the correlation between various parameters (Tables A.14 – A.31 in Appendix).

3.4.1. Physiochemical parameters

The various physiochemical parameters besides secchi depth had little to do with initial pigment concentrations (Table A.14). Secchi depth had significant negative correlation with a few pigment concentrations of the < 20 μm and < 200 μm size fractions, especially fucoxanthin. Therefore, large phytoplankton, specifically diatoms, was related to water turbidity, and most probably a cause for low turbidity. Cell count data on diatom however, did not have significant correlation with secchi depth (Table A.15), most probably because the group includes a large proportion of small cells (See section 3.2.3).

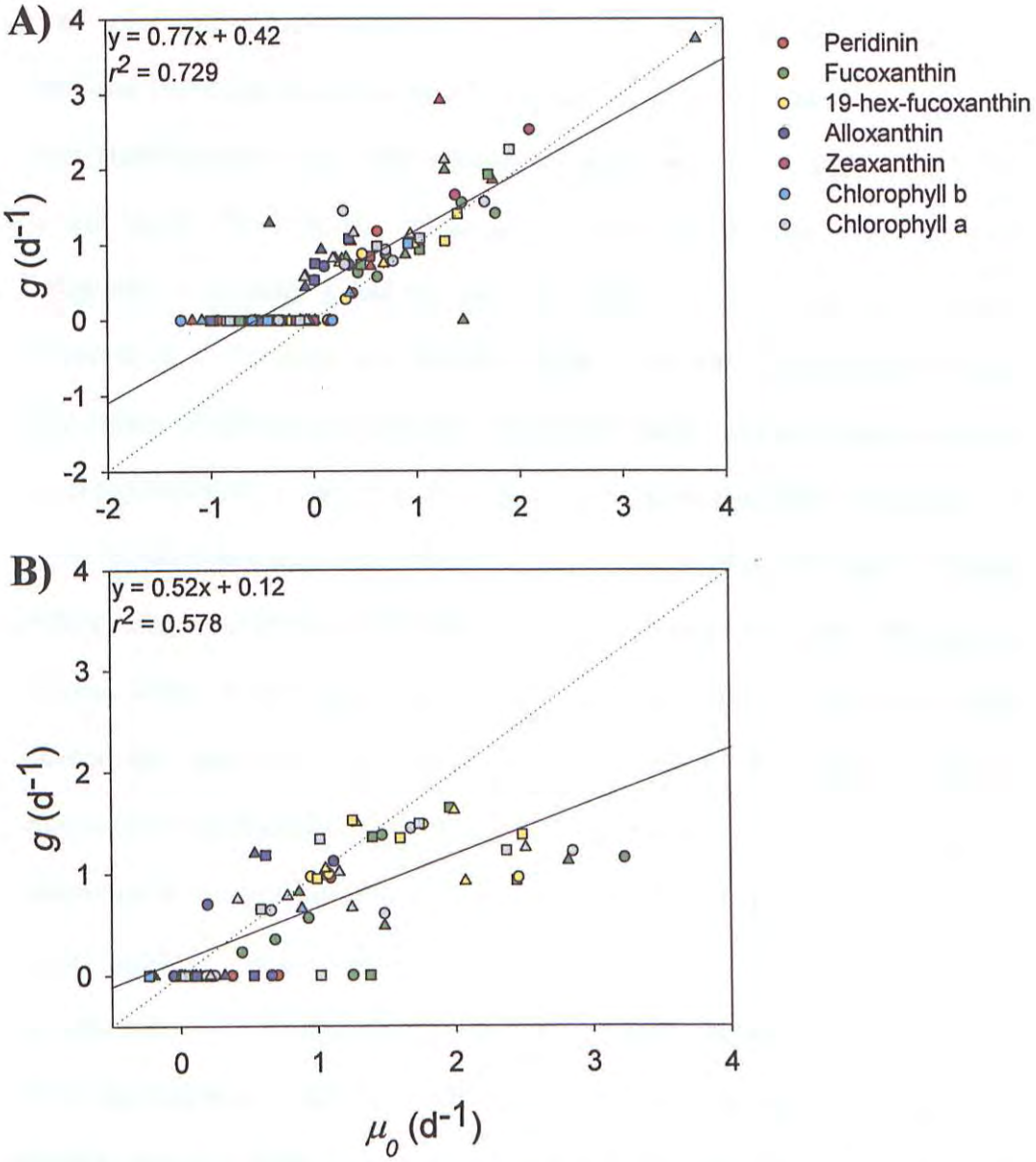


Figure 3.22. The estimated pigment specific microzooplankton grazing rate (g) against the estimated pigment specific phytoplankton growth rate in ambient nutrient (μ_0) of various pigments and size fractions in TH (A) and MB (B). Circle: < 200 μm, triangle: < 20 μm, square: < 5 μm. Solid line: regression curve from data points. Dotted line: equilibrium curve.

Temperature, salinity and dissolved oxygen contents had significant correlations with several μ_n of the < 20 μm and < 200 μm size fractions (Table A.16), which indicates that these parameters may have slight effects on μ_n. Unlike μ_n, μ_0 did not have significant correlation with temperature, salinity, dissolved oxygen content and secchi depth (Table A.17). Nitrite and nitrate concentrations were the most influential to μ_0, since it had the most significant correlation with μ_0 of several pigments of all the three size fractions (Table A.17). This demonstrates the high importance of nitrogenous nutrients, specifically nitrate, and its limitation on the local phytoplankton growth (Lee & Arega 1999, Arega & Lee 2000). Temperature is often regarded as a factor affecting microzooplankton grazing, and often correlated with grazing rates (Peters 1994, Caron et al. 2000, Strom et al. 2001, Obayashi & Tanoue 2002). In this study, temperature, along with salinity, dissolved oxygen content and secchi depth, all had significant correlation with a few g of various pigment and size fractions (Table A.18). Although substantial significant correlation between g on any particular physiochemical parameter was in lacking, as Caron et al. (2000) explains, g at any particular condition are also affected by other factors such as prey and grazer abundance. This implies that although not necessarily strong, these parameters may still have effects on g, and probably on microzooplankton feeding behavior. Ciliates for instance have a wide range of different sensory capabilities, and may therefore be able to respond to different stimuli and environmental conditions (Fenchel & Jonsson 1988). Physio-chemical parameters on the other hand had little effect on the level of microzooplankton grazing control, as indicated by the lack of correlation for many of the physio-chemical parameters with the g/μ_0 ratios of various size fractions and pigments (Table A.19).

3.4.2. Initial pigment concentration

Initial pigment concentration did not have significant correlation with μ_n or g (Tables A.20 and A.21). It may seem at first that g should be related to initial pigment concentrations, since it relates to the encounter rate between grazers and prey. But it should be noted that 1) g is also dependent on the initial phytoplankton standing stock, and it illustrates the rate of removal in terms of the proportion of initial standing stock removed; 2) g is a measure of the community grazing rate instead of individual grazing rates; and 3) grazer densities is somewhat related to initial standing stocks (See section 3.4.3), so that the fraction of standing stock removed can be the same when grazer densities increase with standing stocks. Hence, g can be the same value for different initial pigment concentrations.

< 5 μm alloxanthin had significant correlation to its initial pigment concentration and g/μ_0 ratio (Table A.22). Since the two very high g/μ_0 values of alloxanthin (Figure 3.21) were both from the < 5 μm size fraction, correlation between the < 5 μm alloxanthin initial concentration and microzooplantkon grazing control may therefore be an indication of microzooplankton's high responsiveness towards < 5 μm alloxanthin and thus cryptophyte biomass or densities.

3.4.3. Initial densities

Microzooplankton initial densities had strong correlations with peridinin initial pigment concentration and dinoflagellates cell counts, and alloxanthin initial pigment concentration and cryptophytes cell counts (Tables A.23 and A.24). Since dinoflagellates can be heterotrophic, their correlation may be an autocorrelation effect between two groups of grazers. Correlation with alloxanthin on the other hand, may indicate a preference of microzooplankton towards cryptophyte, which is in correspondence to the results of g/μ_0 ratios (See section 3.4.6).

Besides the obviously expected correlation with peridinin, dinoflagellates initial densities also had significant correlation with 19-hex-fucoxanthin of all three size fractions (Table A.25). This may suggest a preference of dinoflagellates on prymnesiophytes, or the dinoflagellates in our study may have prymnesiophyte endosymbionts that gave them pigment characteristics of 19-hex-fucoxanthin (Jeffrey & Vesk 1997).

Both microzooplankton and dinoflagellates initial densities did not have significant correlation with g (Tables A.27 and A.28).

3.4.4. Phytoplankton growth rates and microzooplankton grazing rates

Most of the μ_n and μ_0 had significant correlation ($p < 0.05$) (Table A.29). Peridinin, zeaxanthin and chlorophyll b however did not exhibit as strong correlation as did other pigments, suggesting that their growth rates may be strongly affected by the addition of nutrients. Another possible reason for the lack of significant correlation may be due to the small sample size (n) these pigments had. Most μ_n and μ_0 had significant correlation with g ($p < 0.05$) (Tables A.30 and A.31). Alloxanthin, despite its rather large sample size ($n = 10$), did not have as high correlation between its μ_n and μ_0 with g compared to other pigment markers. This suggests that cryptophytes were grazed regardless of their growth rates.

3.5. Percentage and composition shifts

3.5.1. Percentage change

Negative percentage changes from initial standing stocks for all pigment markers and size fractions were common in unenriched incubations in TH (Figures 3.23 – 3.29), which may be an indication of nutrient limitation in these incubations. There was no general pattern in terms of percentage changes from initial standing stocks for the three size fractions, but fucoxanthin had high percentage changes compared to other pigments, especially in enriched incubations.

3.5.2. Size fraction

Composition shifts in size were highly variable in both enriched and unenriched incubations (Figures 3.30 – 3.35), and no general pattern can be concluded from size fraction composition shifts. Even though it is suggested that nutrient limitation can lead to an increase dominance of nano- and picophytoplankton (Froneman & Perissinotto 1996a), this was not the consistent case for any pigment in even the most nutrient limited incubations of TH unenriched incubations (See section 3.5.1).

3.5.3. Pigment markers

Composition shifts in pigment markers (Figures 3.36 – 3.41) were relatively less dramatic when compared to shifts in size fractions (Figures 3.30 – 3.35). Incubation may have a stronger effect on pigment markers composition shifts than nutrient enrichment, as most relatively dramatic shifts were between initial and final compositions rather than enriched and unenriched incubations. In cases when there were more obvious differences between enriched and unenriched incubations, it was mostly due to increased composition of fucoxanthin in the enriched incubations. These cases were limited to MB dilution experiments only.

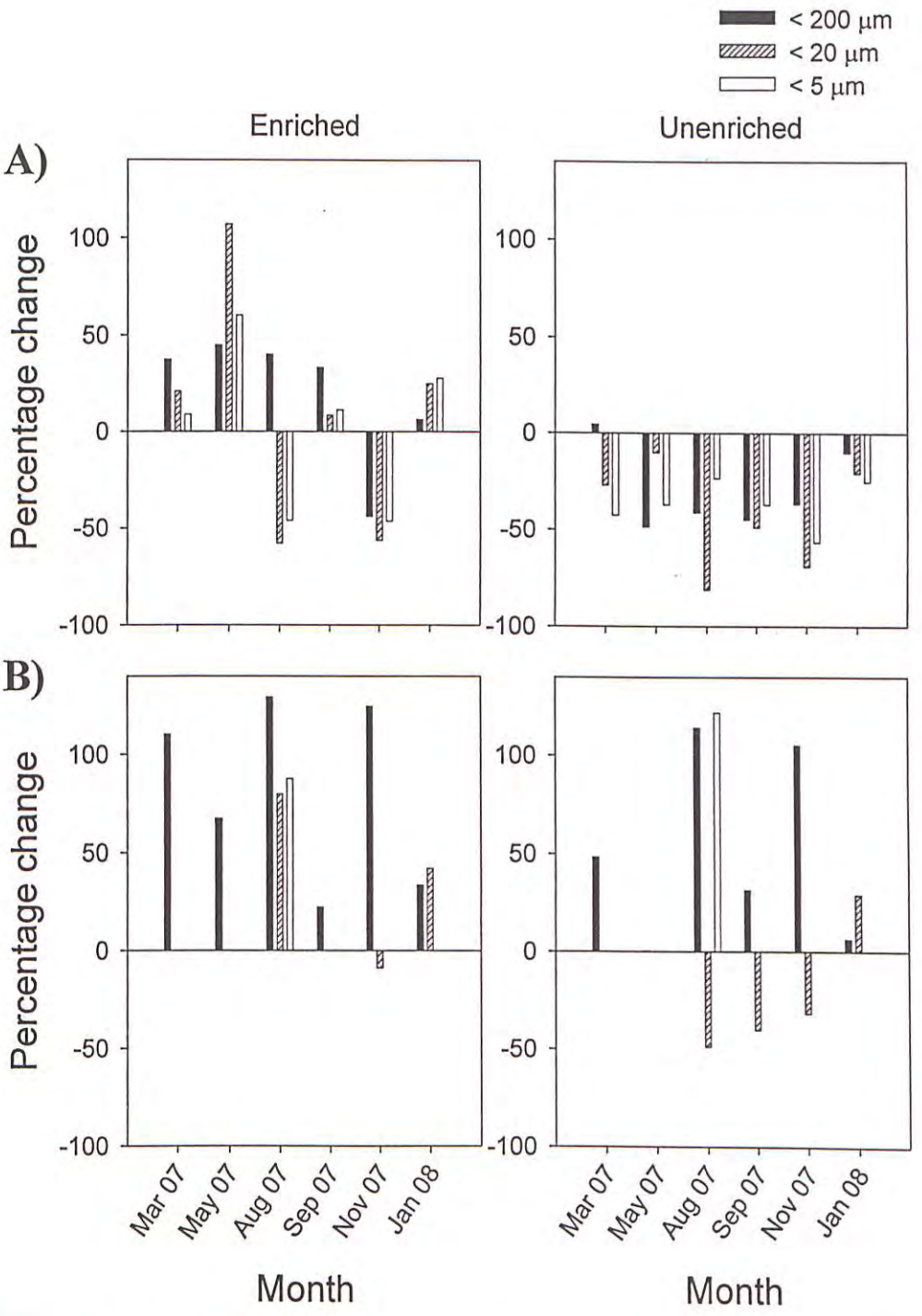


Figure 3.23. Temporal variations in mean percentage changes of < 5 µm, 5 – 20 µm and 20 – 200 µm peridinin concentrations in final enriched and unenriched incubations from the initial standing stock in TH (A) and MB (B) during the study period March 07 – January 08.

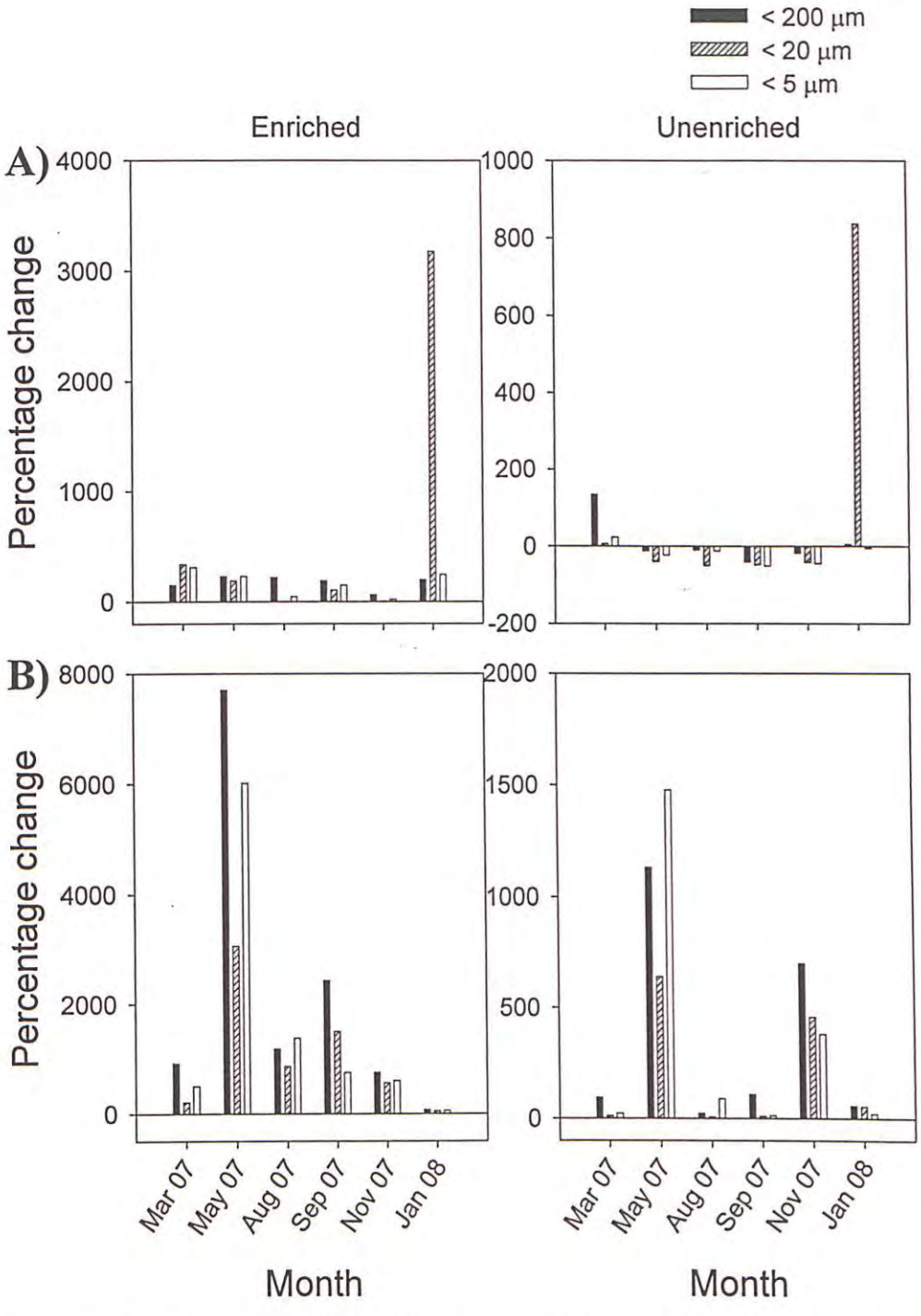


Figure 3.24. Temporal variations in mean percentage changes of < 5 µm, 5 – 20 µm and 20 – 200 µm fucoxanthin concentrations in final enriched and unenriched incubations from the initial standing stock in TH (A) and MB (B) during the study period March 07 – January 08.

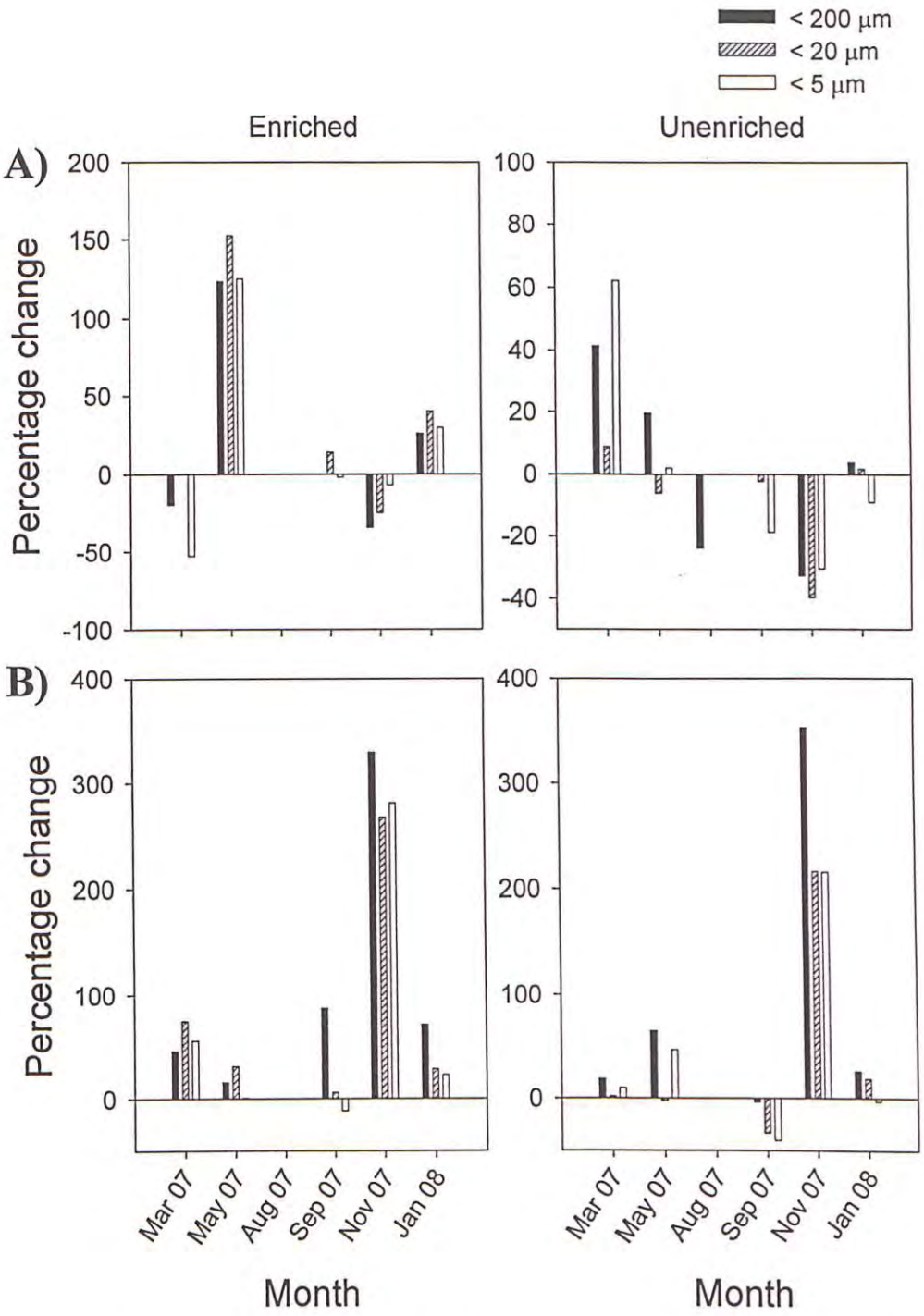


Figure 3.25. Temporal variations in mean percentage changes of < 5 µm, 5 – 20 µm and 20 – 200 µm 19-hex-fucoxanthin concentrations in final enriched and unenriched incubations from the initial standing stock in TH (A) and MB (B) during the study period March 07 – January 08.

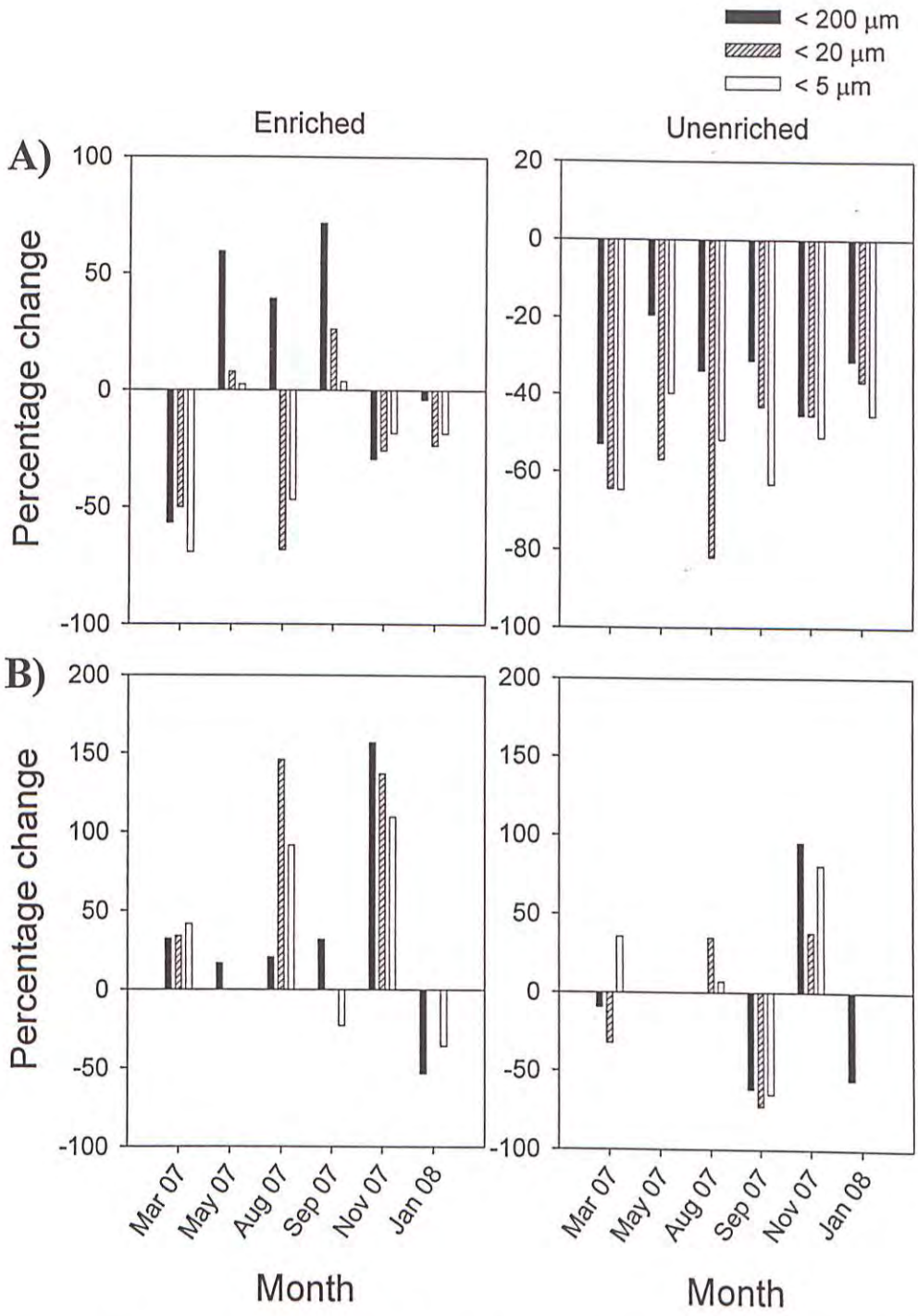


Figure 3.26. Temporal variations in mean percentage changes of < 5 µm, 5 – 20 µm and 20 – 200 µm alloxanthin concentrations in final enriched and unenriched incubations from the initial standing stock in TH (A) and MB (B) during the study period March 07 – January 08.

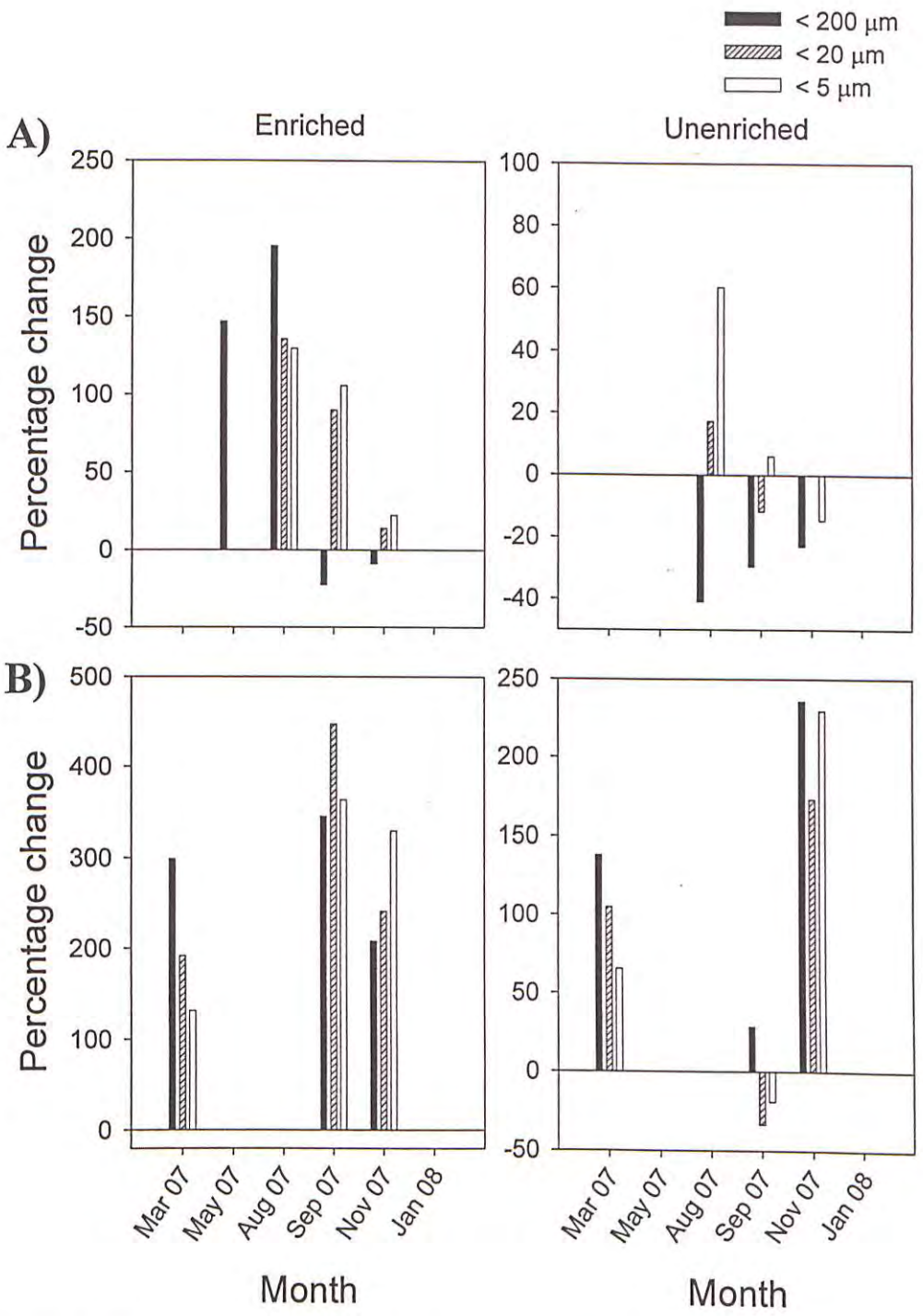


Figure 3.27. Temporal variations in mean percentage changes of < 5 µm, 5 – 20 µm and 20 – 200 µm zeaxanthin concentrations in final enriched and unenriched incubations from the initial standing stock in TH (A) and MB (B) during the study period March 07 – January 08.

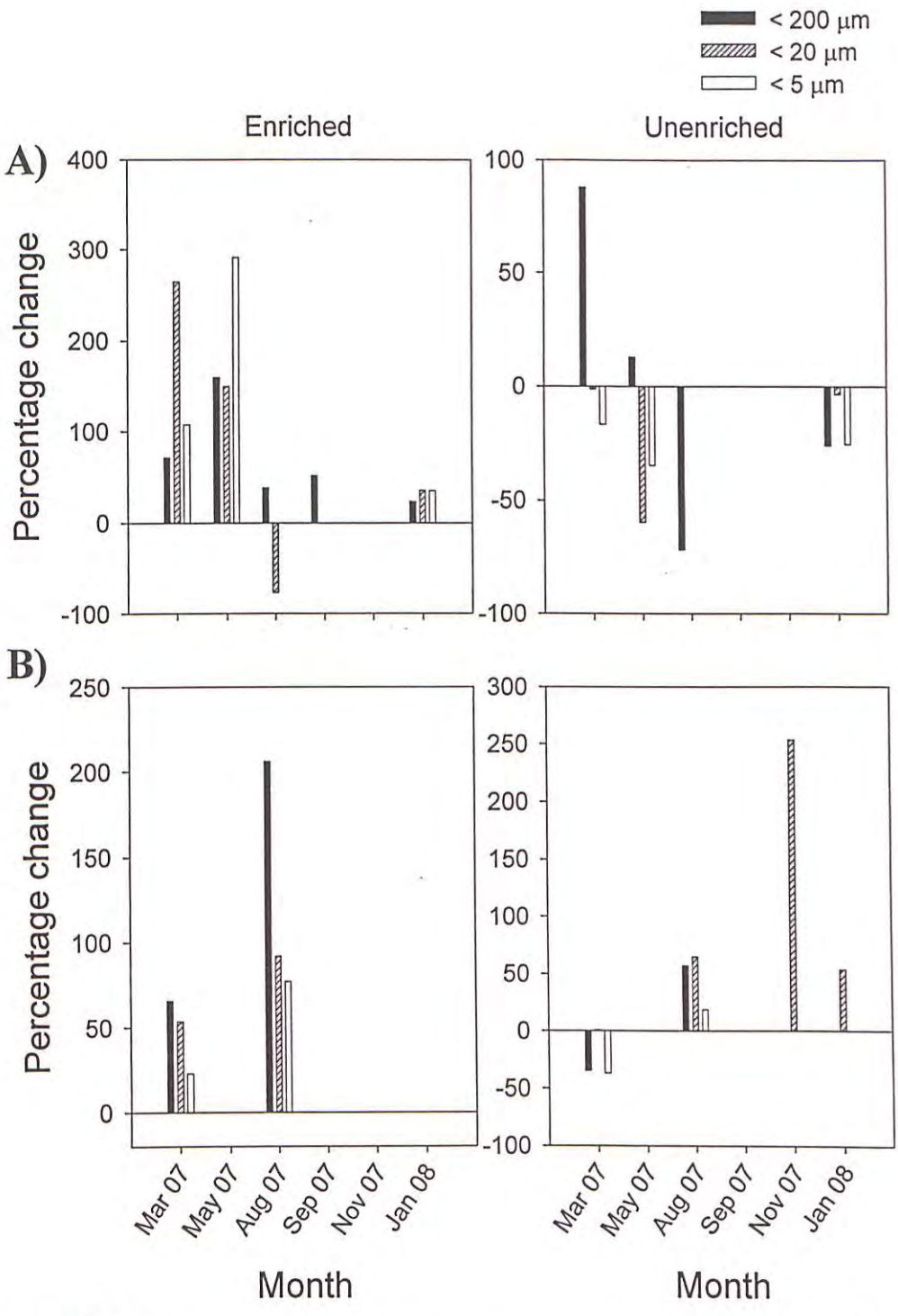


Figure 3.28. Temporal variations in mean percentage changes of < 5 µm, 5 – 20 µm and 20 – 200 µm chlorophyll *b* concentrations in final enriched and unenriched incubations from the initial standing stock in TH (A) and MB (B) during the study period March 07 – January 08.

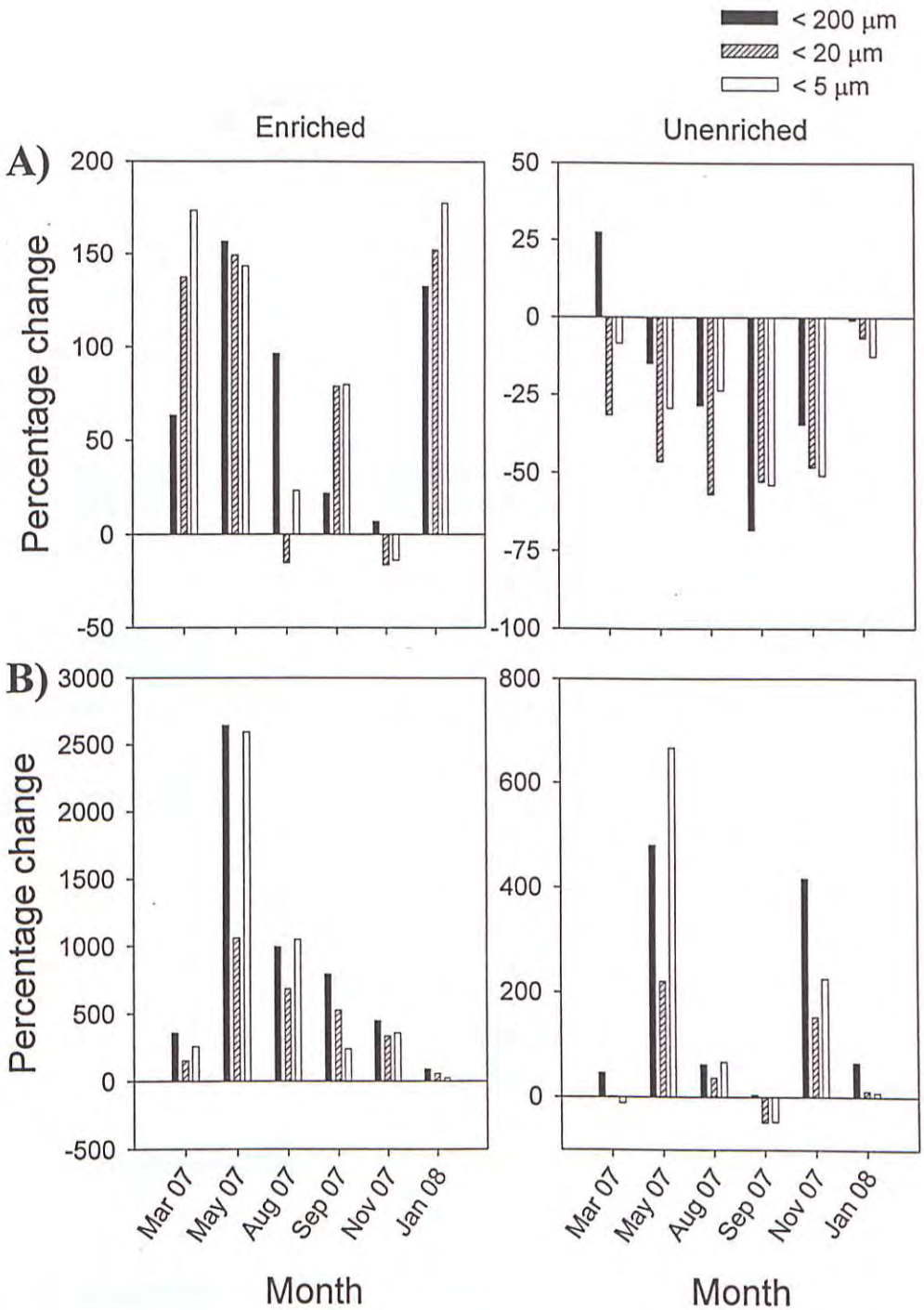


Figure 3.29. Temporal variations in mean percentage changes of < 5 µm, 5 – 20 µm and 20 – 200 µm chlorophyll *a* concentrations in final enriched and unenriched incubations from the initial standing stock in TH (A) and MB (B) during the study period March 07 – January 08.

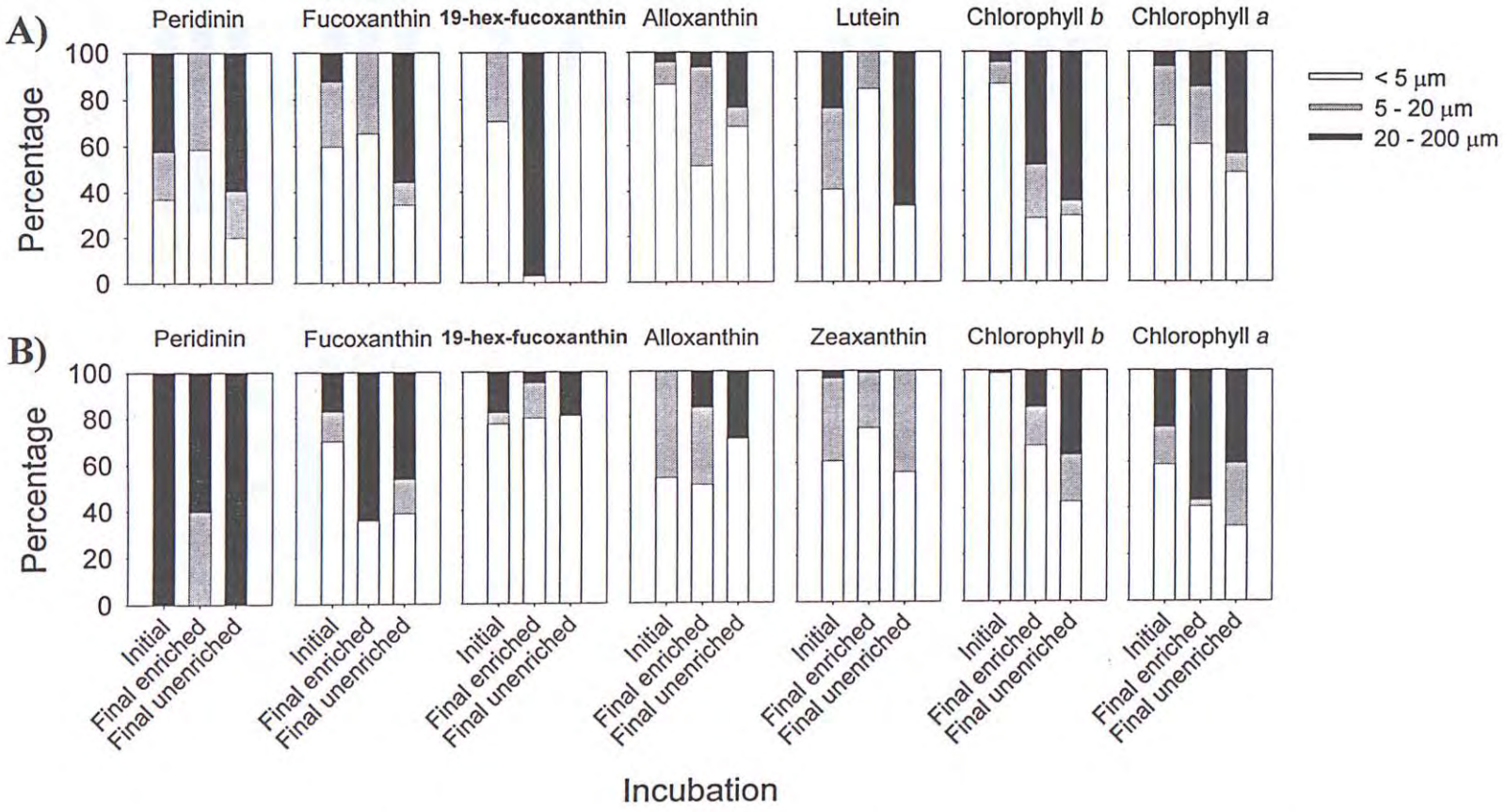


Figure 3.30. Mean percentage of < 5 μm, 5 – 20 μm and 20 – 200 μm various pigments in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B)in March 07.

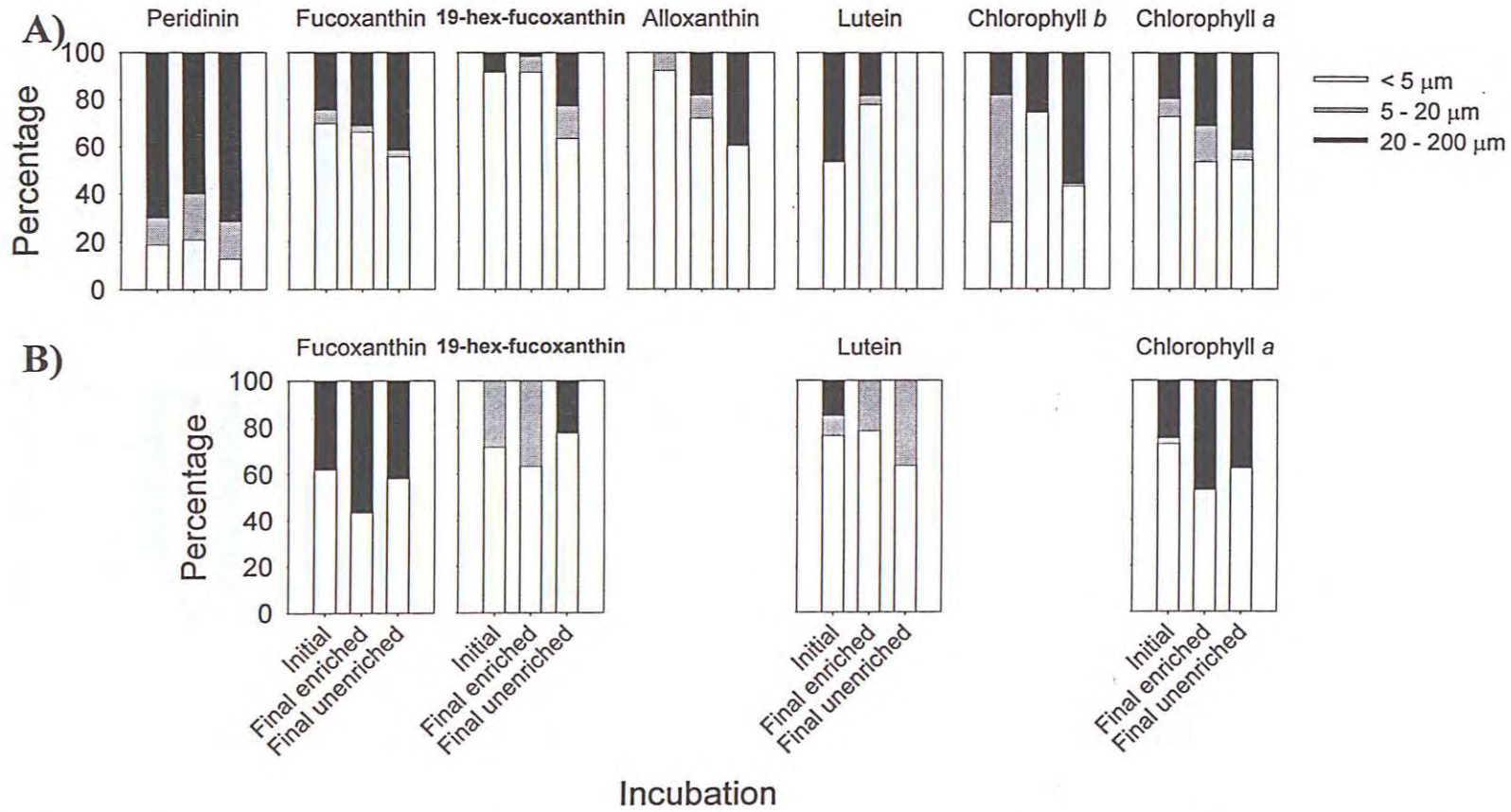


Figure 3.31. Mean percentage of < 5 µm, 5 – 20 µm and 20 – 200 µm various pigments in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in May 07.

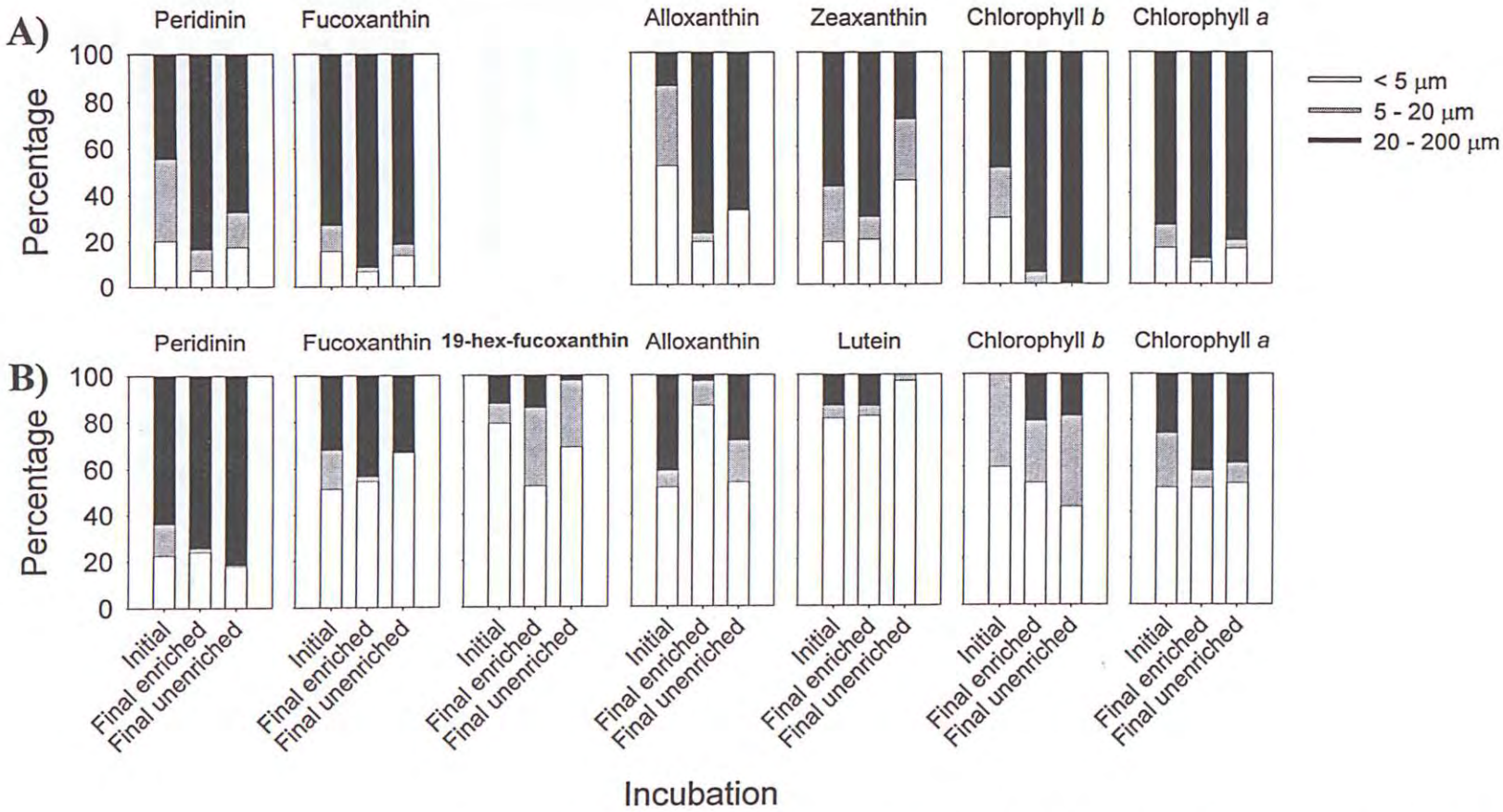


Figure 3.32. Mean percentage of < 5 μm, 5 – 20 μm and 20 – 200 μm various pigments in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in August 07.

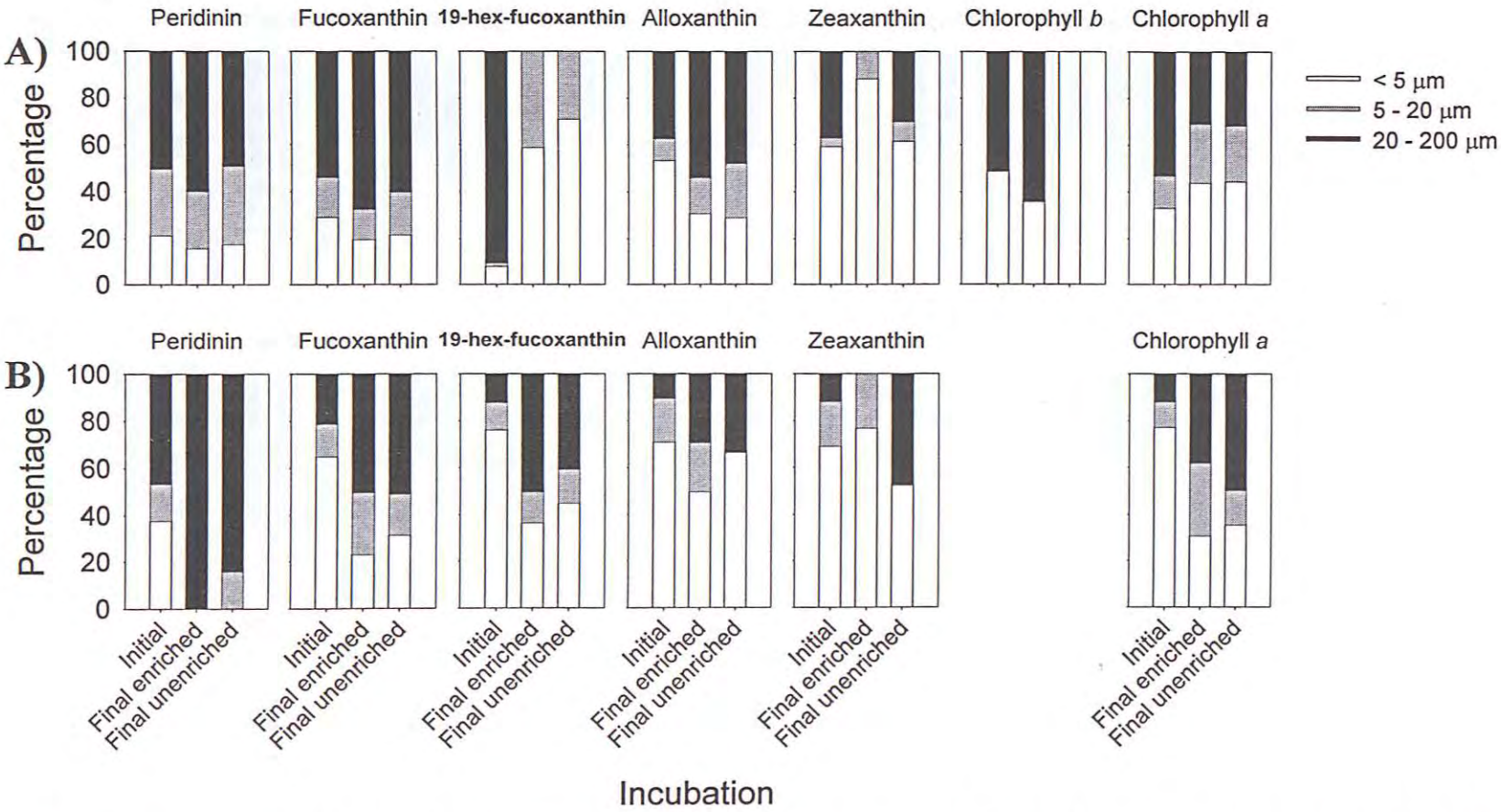


Figure 3.33. Mean percentage of < 5 µm, 5 – 20 µm and 20 – 200 µm various pigments in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in September 07.

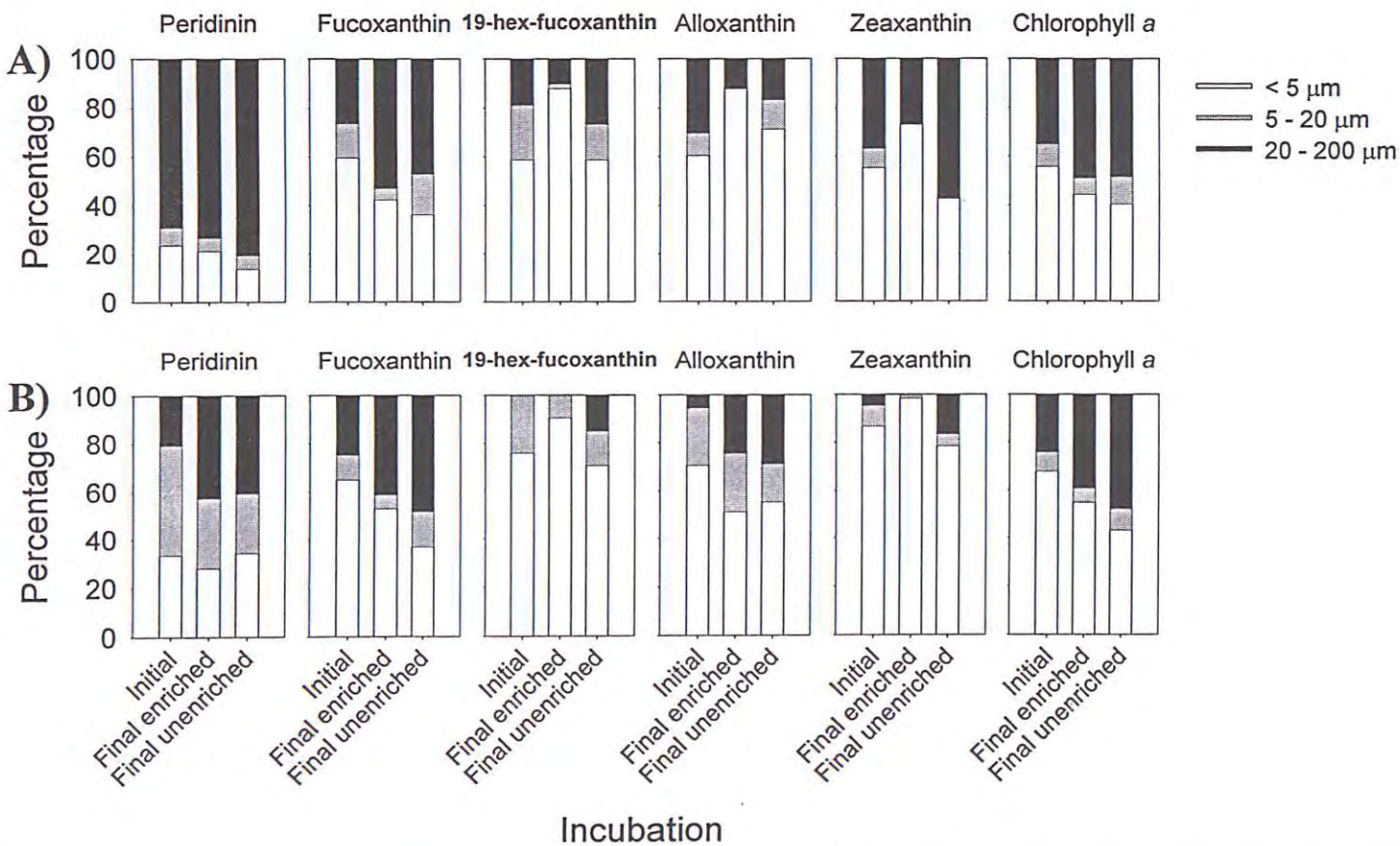


Figure 3.34. Mean percentage of < 5 μm, 5 – 20 μm and 20 – 200 μm various pigments in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in November 07.

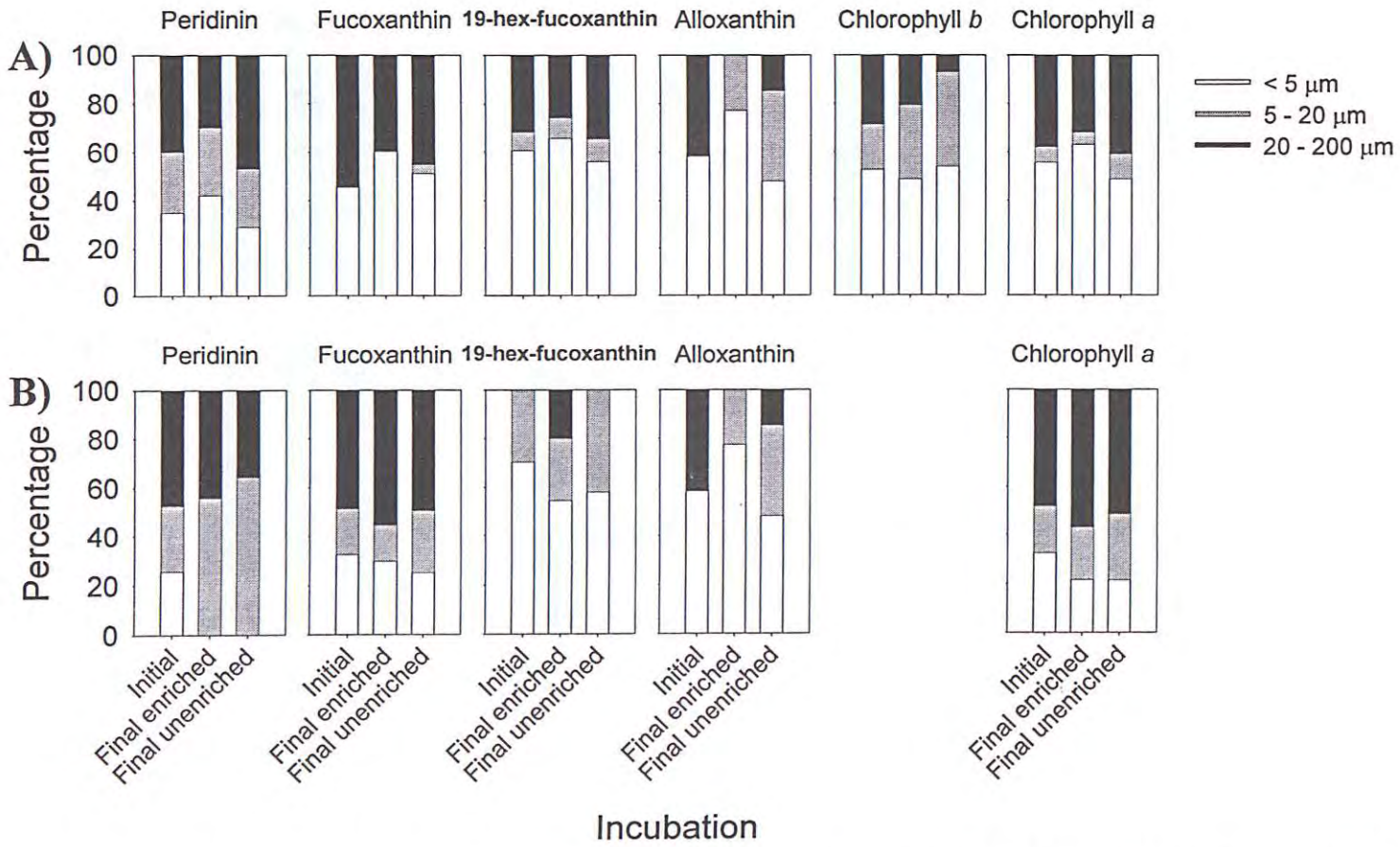


Figure 3.35. Mean percentage of < 5 μm, 5 – 20 μm and 20 – 200 μm various pigments in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in January 08.

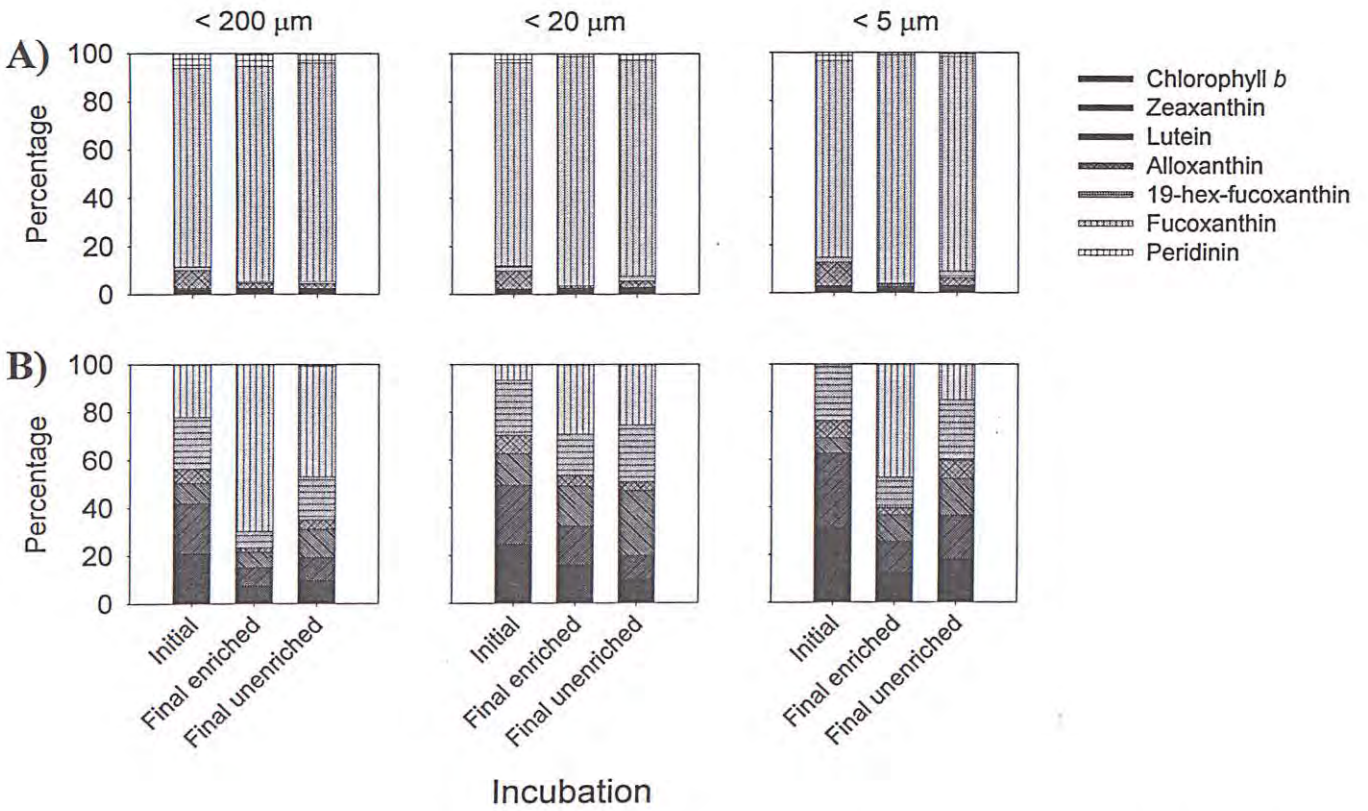


Figure 3.36. Mean percentage of various pigment markers in < 5 µm, < 20 µm and < 200 µm phytoplankton in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in March 07.

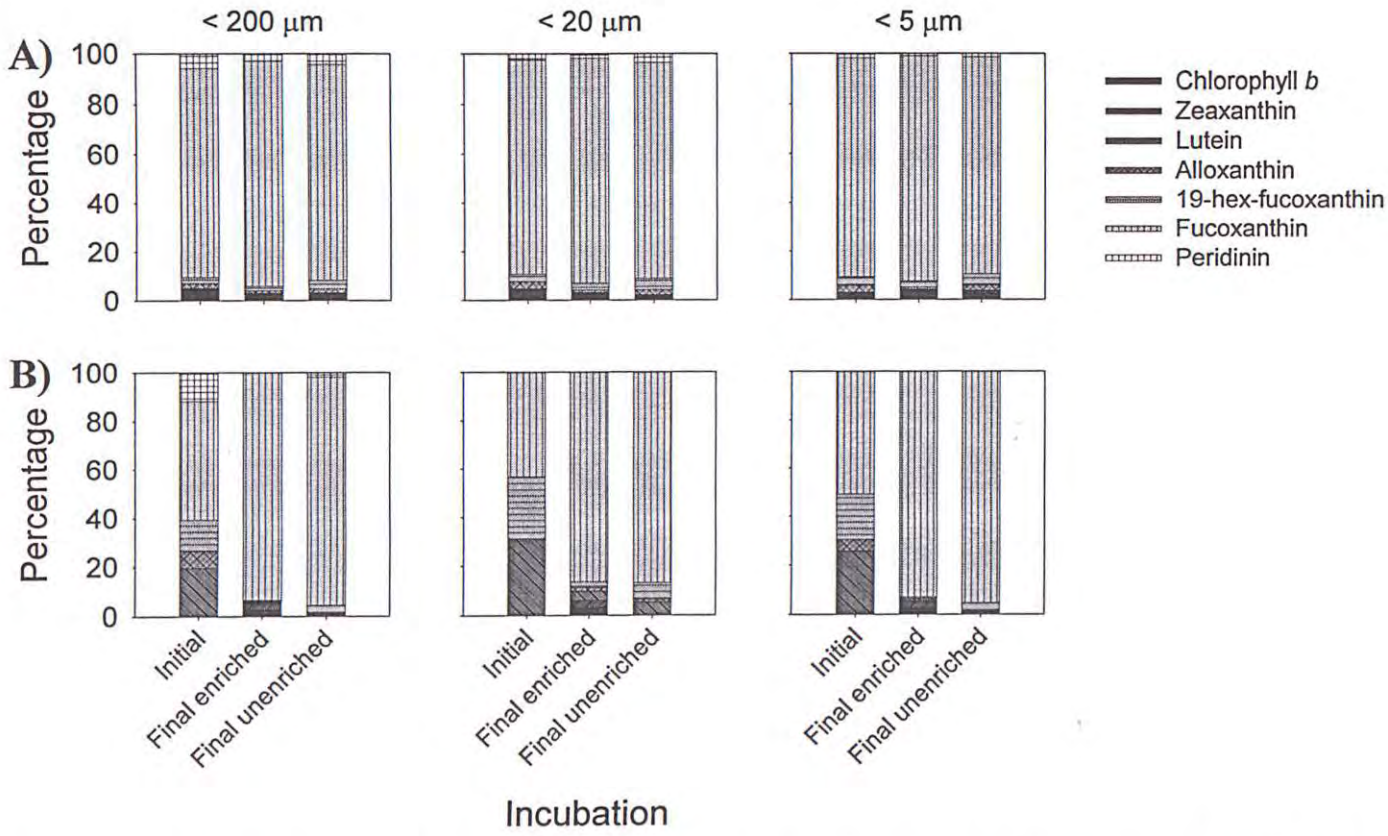


Figure 3.37. Mean percentage of various pigment markers in < 5 µm, < 20 µm and < 200 µm phytoplankton in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in May 07.

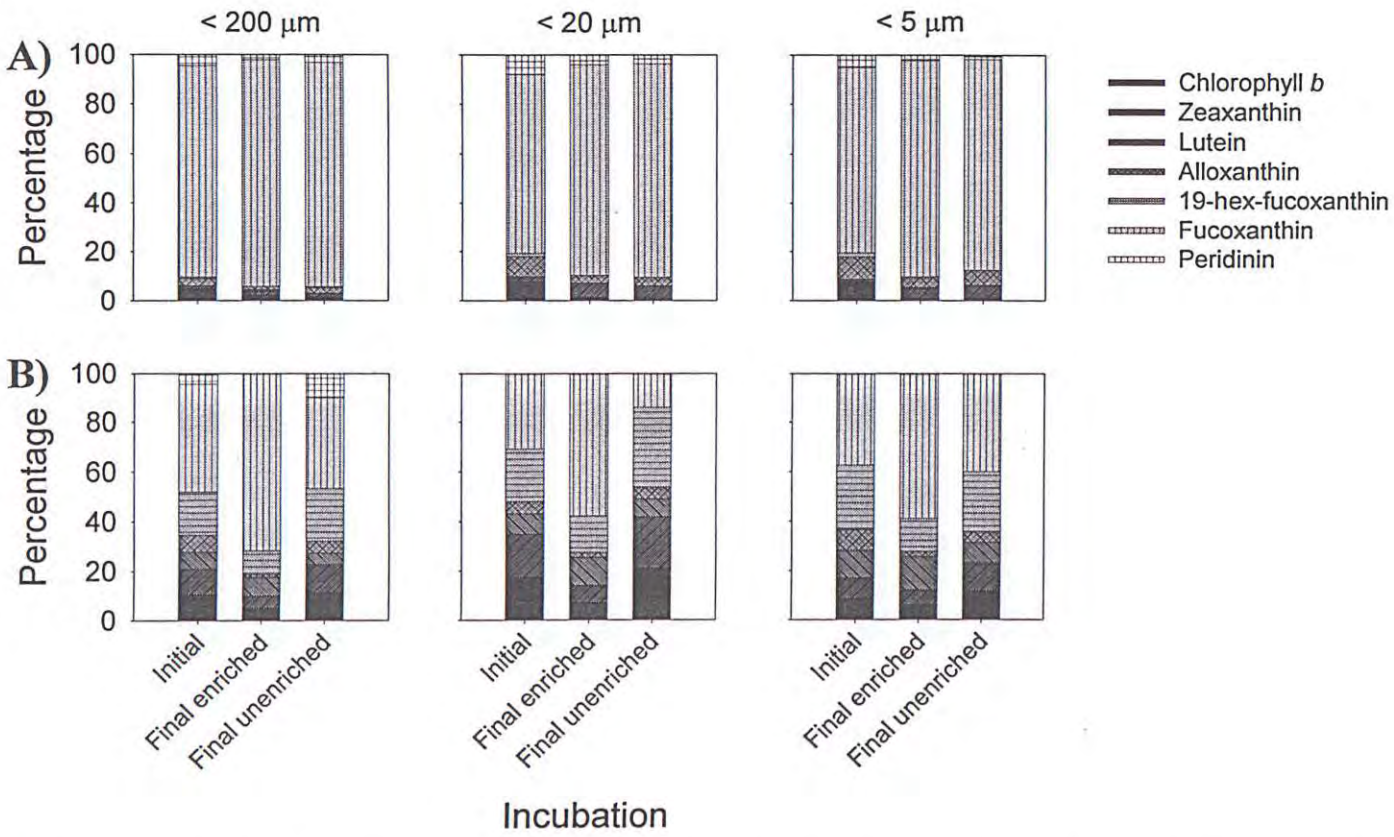


Figure 3.38. Mean percentage of various pigment markers in < 5 µm, < 20 µm and < 200 µm phytoplankton in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in August 07.

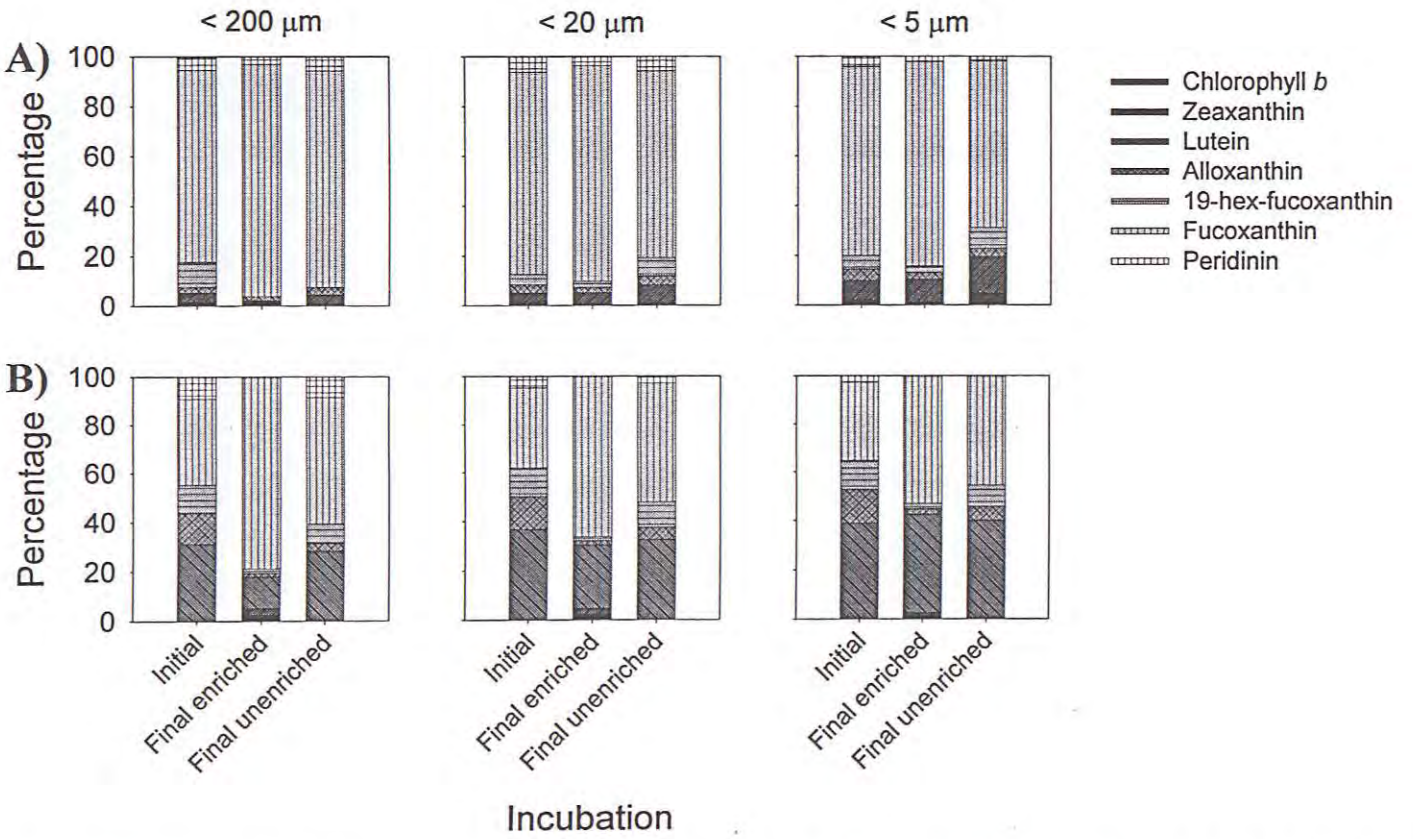


Figure 3.39. Mean percentage of various pigment markers in < 5 µm, < 20 µm and < 200 µm phytoplankton in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in September 07.

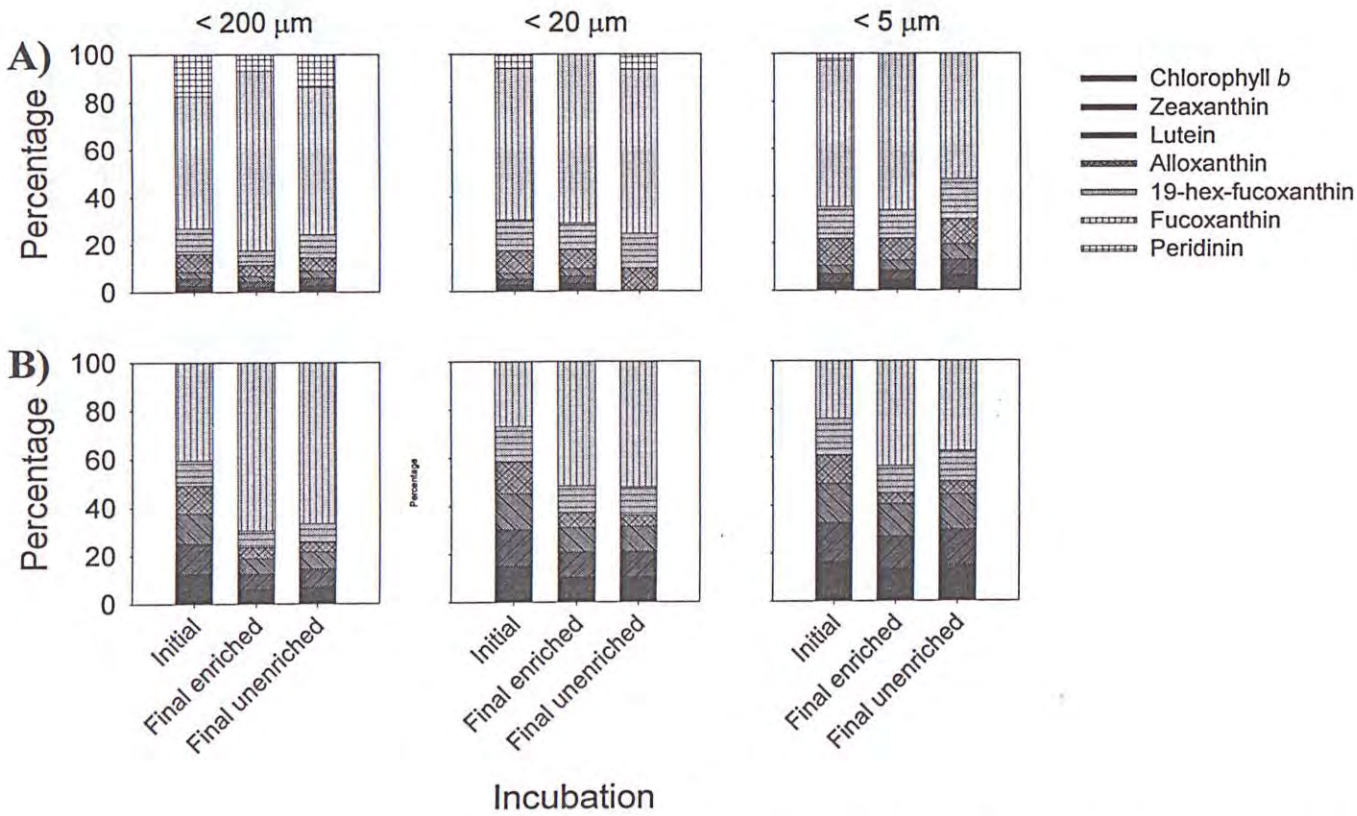


Figure 3.40. Mean percentage of various pigment markers in < 5 µm, < 20 µm and < 200 µm phytoplankton in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in November 07.

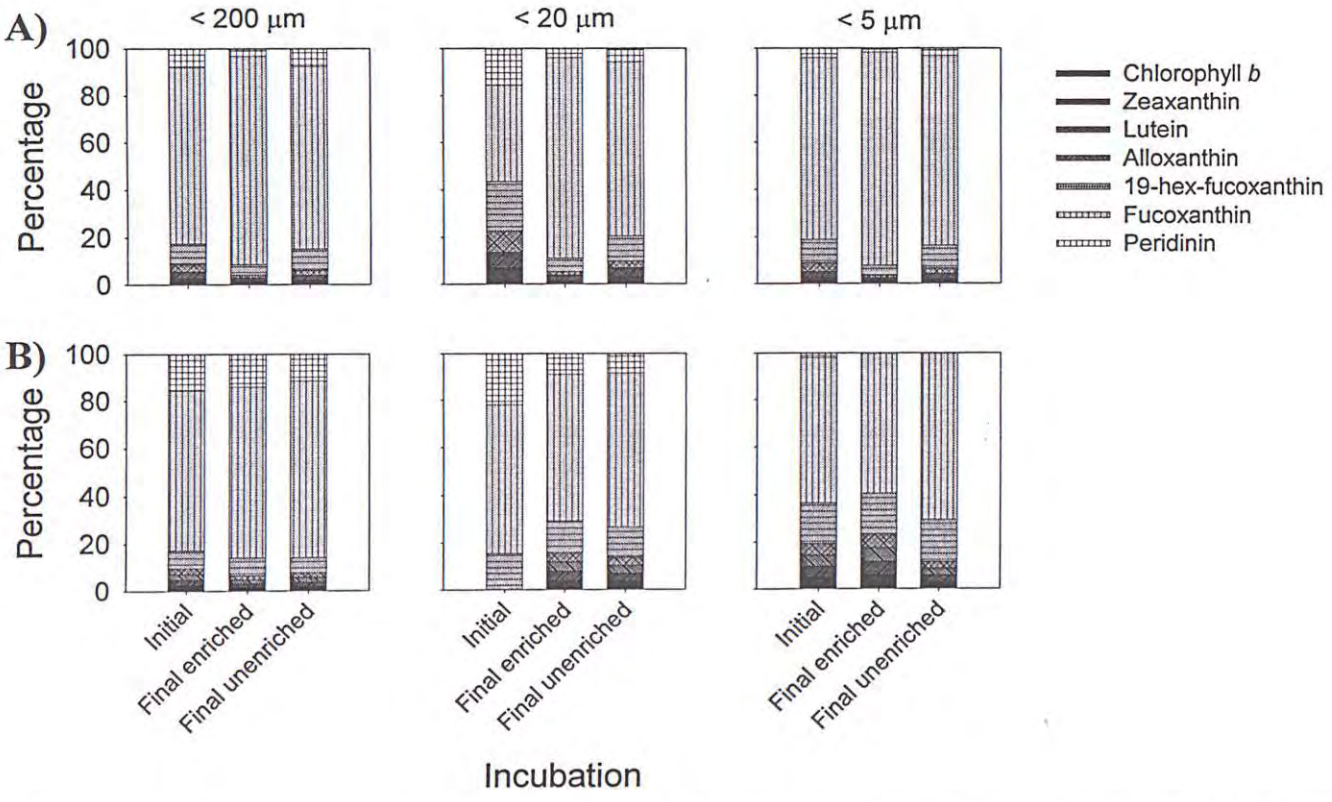


Figure 3.41. Mean percentage of various pigment markers in < 5 µm, < 20 µm and < 200 µm phytoplankton in initial, final enriched and unenriched incubations for the dilution experiments in TH (A) and MB (B) in January 08.

Chapter 4

Discussions

4.1. Hypothesis

Our hypothesis was that the microzooplankton grazing rates (g) would be higher in an area with lower nutrient contents or chlorophyll concentrations because it will contain a higher proportion of small phytoplankton. However, our results showed that even though chlorophyll *a* concentrations were lower in MB than in TH throughout the whole study period, the proportion of phytoplankton in the < 5 μm size fraction and microzooplankton grazing rates (g) were not higher in MB. In addition, our results do not show size selectivity feeding by microzooplankton (See section 4.4.1.), which was our basis to believe microzooplankton grazing rates would be higher in areas with smaller phytoplankton. Our experiment therefore could not test our hypothesis. There was a brief period in the summer (August and September) when the proportion of < 5 μm phytoplankton was indeed higher in MB. But even during this period, g remained comparable between the two sites. The hypothesis is therefore not supported by our results.

While g was not higher in MB than in TH, g/μ_0 ratios were higher in TH generally throughout the whole study period, even during the summer of lower proportions of < 5 μm phytoplankton in TH, the ratios were still higher in TH. This may be because of the lack of selectivity towards phytoplankton size we found in the microzooplankton community in this study (See section 4.4.1.).

It is not clear why our results did not fit the general rule regarding higher compositions of smaller phytoplankton in lower chlorophyll contents ecosystems (See section 1.4.3). Nutrient levels were probably too high even in MB to shift the phytoplankton community towards small cells (Figure 3.2). According to the three dimensional growth rate model of Parsons and Takahashi (1973), at the high light

intensity of 0.10 ly min^{-1}, 2.2 μM concentration of nitrogen will be enough for the large phytoplankton *Dithylum brightwellii* to grow faster than the smaller phytoplankton *Coccolithus huxleyi*. The mean concentration of nitrate and nitrite alone throughout the entire study in MB was already 5.76 μM, so it is possible that large phytoplankton growth was not at a disadvantage in MB. In addition, studies reporting small phytoplankton dominance in oligotrophic systems were mostly conducted in open oceans, and so the feature may be limited to open oceans only (e.g. Le Bouteiller et al. 1992, Blanchot & Rodier 1996, Marañón et al. 2000). To our knowledge, there is no previous study or data on size fractionated chlorophyll concentrations in both sites to compare our results with. One cause of the phenomenon that we speculate was selective feeding on larger phytoplankton cells by mesograzers excluded from this study. Since most mesograzers' prey size range is limited by the morphology of their feeding apparatus and mechanisms (Hansen et al. 1994, Calbet & Landry 1999), they may feed selectively on larger particles. While the northeastern waters of Hong Kong are reported to be dominated by small copepod genera such as *Paracalanus* and *Oithona* (Tang et al. 1994), these genera have been shown to be able to feed on or even select > 20 μm food particles (Paffenhöfer 1984, Tsuda & Nemoto 1988, Castellani et al. 2005).

4.2. Phytoplankton growth rates and microzooplankton grazing rates

Although negative phytoplankton growth rates are rare in most dilution experiments, they are commonly reported (e.g. Strom & Strom 1996, James & Hall 1998, Kim et al. 2007). Since the negative phytoplankton growth rates in our study appear mostly in unenriched incubations, we speculate they were mainly due to nutrient depletion. Peridinin produced some negative μ_n, especially in TH. But dinoflagellates, indicated by peridinin, may be heterotrophic instead of

photoautotrophic, especially in warm tropical waters (Jeffrey & Vesk 1997), and may not benefit from the addition of nutrients. Compared to other dilution experiments carried out in subtropical or coastal waters, our range of -0.17 – 2.87 d^{-1} and 0.00 – 2.26 d^{-1} fit into the reported range of -0.63 – 3.41 and 0.00 – 3.86 d^{-1} for μ_0 and g respecitively (e.g. Strom & Strom 1996, Landry et al. 1998, Ruiz et al. 1998, Landry & Calbet 2004, Kim et al. 2007, Palomares-García et al. 2007). In August, our g of 2.26 d^{-1} in TH and 1.48 d^{-1} in MB for < 200 μm chlorophyll *a*, were higher than the g of 0.71 d^{-1} and 0.56d^{-1} reported by Sun et al. (2003) for two different sites at the east- and western areas of Hong Kong in August 2000.

Both the SS and Production grazed obtained in this study were higher than the averaged value summarized by Calbet and Landry (2004) for our ecosystem, which were ~ 47.3% and 55.1% for coastal and tropical ecosystems respectively for chlorophyll *a* SS grazed, and ~ 59.9% and 74.5% for coastal and tropical ecosystems respectively for chlorophyll *a* Production grazed. While the average chlorophyll *a* Production grazed for total phytoplankton (TH: 155.9% and MB: 93.1%) in this study seems high compared to Calbet and Landry's averaged data, the maximum values (TH: 319.2% and MB: 155.4%) (Tables A.8 – A.13 in Appendix) were still lower than results obtained in previous dilution experiments from e.g. Safi et al. 2007 (Maximum value: 513%) and Zhang et al. 2005 (Maximum value: 468%).

4.3. Dilution experiment

4.3.1. Nutrient enrichment

Although nutrient enrichment is often employed to satisfy the assumption that phytoplankton growth is not limited by nutrients (See section 1.2.1), recently more and more studies seem to abandon this procedure (e.g. Kuipers & Witte 1999, Strom et al. 2001, Obayashi & Tanoue 2002, Palomares-Garcia et al. 2006, Zhang et al.

2006, Safi et al. 2007) when it was assumed that ambient nutrients were sufficient. The reluctance for nutrient additions may possibly due to the fear of affecting the microzooplankton community (Gifford 1988) by such addition.

In this study, preliminary experiments indicated the need for nutrient enrichment (See section 2.2.1) and although μ_n and μ_0 were similar in most cases, the fact that there were more negative μ_0 than μ_n and that they were usually lower than μ_n justifies the need for nutrient enrichment. Peridinin produced some negative μ_n, especially in TH. But some dinoflagellates may be heterotrophic instead of photoautotrophic, especially in warm water tropical waters (Jeffrey & Vesk 1997), and may not benefit from nutrient addition.

4.3.2. Shift of pigment compositions

Besides affecting the microzooplankton assemblage (See section 1.2.3 and 4.1.1), nutrient enrichment or incubation (Strom & Welschmeyer 1991) may also affect the phytoplankton assemblage. Yet shifts in phytoplankton community composition, whether size or groups, are rarely investigated.

Composition shifts in size fractions were so variable that no conclusion can be made on whether nutrient addition had caused any effect. However, increased composition of fucoxanthin can occasionally be found in MB enriched incubation. This leads to the speculation that nutrient enrichment may induce more diatom growth in incubations with low chlorophyll concentrations. Group compositions were more stable in TH. We speculate that this resistance to group composition changes may be in part due to the higher phytoplankton density found in TH, or an intrinsic acclimation to high nutrient contents from a long history of eutrophication (See section 1.8.1)

4.3.3. Experiment limitations

The three different size fractions were obtained from three identical 1.2 L replicas instead of from the same incubation. This was due to a limitation in equipment, since if we were to take all HPLC samples for the three different size fractions from one incubation, then the incubation bottle would need to hold at least 3 L, which would be difficult to handle at such size. This in part contributes to the presence of alloxanthin and zeaxanthin, which are generally considered to be < 20 μm, to appear in the 20 – 200 μm size fraction obtained by subtracting < 20 μm concentrations from < 200 μm concentrations. The occurrence of large phytoplankton, such as dinoflagellates, in small size fractions on the other hand is due to the fact that the maximum dimension of particles does not neccessarily determine retention. When water flows through the meshes, particles may line up longitudinally so that only their width affects retention (Sieburth et al 1978).

Due to limitation in manpower, equipment and space, the dilution experiment for each site had to be performed on separate dates. We realized that it is inaccurate to compare between the results when the experiments were performed on separate dates, but the work required to perform one complete set of dilution experiment is so intensive that it was just impossible to do two set of dilution experiments for both sites on the same date. In addition, space and equipment available were also limited for just one complete set of dilution experiment as well.

Estimating g was difficult when pigment concentrations were low (See section 3.3.1). This was a limitation in the sensitivity of our HPLC machine. If pigment concentrations were to be higher, more significant g estimations might have been made. But again due to the volume of incubation bottles and the need to retain a small volume of sample for microscopic cell counts, only about 1 L of water that can be used for HPLC sampling, which was often insufficient when pigment

concentrations were low. As pigment concentrations in MB were often low, incubation period for MB dilution experiments was changed to 48 h instead of 24 h in the hopes of increasing the differences between initial and final pigment concentration for easier detection.

4.4. Microzooplankton feeding preference

4.4.1. Phytoplankton size

Results of the few previous dilution experiments considering different phytoplankton size fractions are not in agreement. While Froneman and McQuaid (1997) and Zhang et al. (2005) reported slightly higher grazing rates on smaller phytoplankton size fractions, Strom et al. (2001) and Safi et al. (2007) did not find such pattern. Our own results also did not show any substantial evidence for food size preference by microzooplankton as well. Since the *g* obtained in this study were for < 200 μm, < 20 μm and < 5 μm, instead of 20 – 200 μm, 5 – 20 μm and < 5 μm (See section 3.3.1 for reason), *g* of different size fractions cannot be compared directly because the larger size fractions also includes the smaller ones. However, if *g* on the smaller size fractions were indeed higher, than it is expected that < 5 μm *g* would be higher than the other two size fractions since the proportion of smaller cells removed by grazing will be higher. Although < 20 μm and < 5 μm *g* were often higher than < 200 μm, that is not the case consistently. Considering grazing on chlorophyll *a*, of the 12 sets of dilution experiments conducted in the two sites, 4 sets had the highest *g* on < 5 μm, but 6 sets had the lowest *g* on < 5 μm, though most of these cases *g* were assumed to be 0 due to failed regression analyses (Figure 3.20).

Froneman and Perissinotto (1996a, 1996b) used another method to study size fraction preference in microzooplankton dilution experiments, in which they compared the percentage change of initial stock of different size fractions, and found

that nanophytoplankton and picophytoplankton usually had negative percentage changes. However, when our own data were presented this way (Figures 3.23 – 3.29), we again did not find any consistent patterns towards different size fractions percentage changes. Therefore, we cannot conclude that there were preferences towards smaller size fractions.

4.4.2. Phytoplankton group

The g/μ_0 ratios for alloxanthin were high for both sites, with all values (except in cases when g was assumed to be 0) exceeding 1, and extremely high values found in TH (Figure 3.21). This may be an indication of preference towards cryptophytes by our local microzooplankton. Cryptophytes were also found to be strongly preferred by *Penilia avirostris* in Tolo Harbour (Wong et al. 2006). So the local cryptophytes may be subjected to high grazing impacts from the local microzooplankton community, *P. avirostris* and possibly other mesozooplankters. It was not surprising to find that cryptophytes are such preferred prey since there have been reports on dinoflagellates preference towards cryptophytes as a source of kleptochloroplast (Eriksen et al. 2000). Even the once considered exclusive photosynthetic ciliate *Mesodinium rubrum* have been found to feed on cryptophytes (Gustafson et al. 2000, Yih et al. 2004). To our knowledge however, no previous dilution experiments have reported preference on cryptophytes.

Although there is no agreement on microzooplankton preference towards a certain phytoplankton group in previous dilution experiments, it is often reported that these grazers prefer fast growing phytoplankton groups, regardless of whether these groups are the most abundant (Burkill et al. 1987, Strom & Welschmeyer 1991, Gaul & Antia 2001, Strom 2002). Our data supports the pattern of g increasing with μ_0 in both sites (Figure 3.22), and that μ_0 of various pigments and size fractions had

significant correlations with *g*, (Table A.31). Several explanations are suggested for this phenomenon, such as higher grazer production in response to higher prey production and increased grazers feeding activities (Strom 2002). But it is often suggested that this is the result of a shift in microzooplankton grazing preference, in response to the increased prey abundance (if the prey abundance was indeed increased) or nutritional value (Strom 2002). Nevertheless, such correlation is an indication of the behavioral capabilities of microzooplankton to quick responses to changes in phytoplankton growth rates, allowing the tight coupling between phytoplankton growth rates and microzooplankton grazing rates (e.g. Strom & Welschmeyer 1991, Kim et al. 2007, Safi et al. 2007).

4.5. Food web dynamics

4.5.1. The role of microzooplankton

4.5.1.1. Nutrient recycling

As mentioned in section 1.3, whether nutrients are recycled or exported is dependent on both the phytoplankton size and microzooplankton size selectivity in the ecosystem. In both of our study sites, the phytoplankton communities were often dominated by picophytoplankton, and microzooplankton were found to have no size preference. Most nutrients in these two ecosystems would therefore be recycled, especially when the microzooplankters are such major consumers of phytoplankton, as indicated by the high SS and Production grazed (Tables A.8 – A.13). Part of the remaining stock of phytoplankton might also be grazed by mesozooplankters, so the chance for nutrient export through phytoplankton sinking is low.

Efficient nutrient recycling and uptake by phytoplankton might be the reason why production in TH were so high, as indicated by the chlorophyll *a* concentrations (Figure 3.3), when ambient dissolved nutrient levels were not particularly high

(Figure 3.2).

4.5.1.2. Energy transfer

An important role of microzooplankton in the food web is to transfer energy in phytoplankton to higher trophic levels (See section 1.3). From the results of higher SS and Production grazed in TH (Tables A.8 – A.13), and the knowledge that TH has higher densities of mesozooplankton than MB (Wong unpublished data), microzooplankters may have a heavier role in energy transfer to higher trophic levels (i.e. mesozooplankton) in TH than in MB.

4.5.1.3. Phytoplankton control

Judging from the g/μ_0 ratios, SS and Production grazed (Figure 3.21, Tables A.8 – A.13), the microzooplankton communities in both sites, especially in TH, were capable of controlling the phytoplankton communities. Since all g/μ_0 ratios, SS and Production grazed were found to be much higher in TH, one might expect that phytoplankton densities to be lower in TH due to the higher grazing pressure. On the contrary, TH had much higher phytoplankton production than MB (Figure 3.3) which implies that the *in-situ* grazing pressure in TH should be much more reduced, most probably due to the removal of micrograzers by mesozooplankton selective feeding (See section 1.3).

4.5.2. The role of mesozooplankton

Dilution experiments are preformed conventionally with the exclusion of mesozooplankton to study solely the feeding of grazers smaller than 200 μm. However, it is often considered that mesozooplankton affects the microbial food web by selective feeding on microzooplankton (See section 1.3). The exclusion of

mesograzers also does not allow accurate evaluation of the balance between growth and grazing in large species of phytoplankton as well (Strom & Welschmeyer 1991). It would therefore be interesting to investigate further the *in-situ* food web dynamics by performing simultaneous dilution experiments with and without mesozooplankton, and then compare grazing mortality on phytoplankton. If indeed mesozooplankton has selective feeding on microzooplankton, phytoplankton grazing mortality rates would be expected to decrease due to the reduced grazing pressure through the removal of micrograzers. On the other hand, if mesozooplankton fed selectively on phytoplankton instead, than phytoplankton grazing mortality rates would increase due to the increased grazing pressure exerted by the addition of mesograzers.

Chapter 5

Conclusions

This study is the first to investigate microzooplankton grazing in Tolo Harbour and Mirs Bay. The hypothesis that Mirs Bay has lower nutrients and higher proportions of small phytoplankton and therefore higher grazing rates could not be tested because proportion of small phytoplankton were similar between the two sites. Results showed that Mirs Bay did not have lower nutrients, higher proportions of small phytoplankton, or higher grazing rates consistently. It was also found that microzooplankton in both sites did not have any size preference towards phytoplankton, but may have preferences towards cryptophytes, which were found to be grazed heavily despite their low growth rates.

Phytoplankton growth rates and microzooplankton grazing rates were found to be comparable between Tolo Harbour and Mirs Bay, but the microzooplankton grazing impact was higher in Tolo Harbour, which may indicate that the microzooplankton there play a heavier role in transferring phytoplankton production up higher trophic levels.

Although Tolo Harbour had much higher chlorophyll concentrations than Mirs Bay, it defied the conventional belief that higher chlorophyll contents leads to lower proportions of small phytoplankton. Algal blooms in Tolo Harbour were found to be caused mainly by an increase in the abundance of small phytoplankton.

Dilution experiments proved to be a convenient way of estimating microzooplankton grazing simultaneously with phytoplankton growth rates. However, microzooplankton grazing rates were difficult to estimate when phytoplankton pigment concentrations and possibly grazing rates were low. It was found that nutrient enrichment to the incubations may lead to changes in community

composition in certain samples.

The exclusion of mesozooplankton in dilution experiments may lead to inaccurate estimations of *in-situ* phytoplankton grazing mortalities. And it may be interesting to compare phytoplankton grazing mortalities in dilution experiments with and without mesozooplankton.

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Table A.1. List of abbreviations used in this study for chemotaxonomic pigment markers (A) and other pigments detected (B).

A)

Pigment	Abbreviation
Peridinin	Peri
Fucoxanthin	Fuco
19-hex-fucoxanthin	19 hex
Alloxanthin	Allo
Zeaxanthin	Zea
Chlorophyll *b*	Chl *b*
Chlorophyll *a*	Chl *a*

B)

Pigment	Abbreviation
Chlorophyll c_3	Chl *c3*
Chlorophyll c_2	Chl *c2*
19-but-fucoxanthin	19 but
Prasinoxanthin	Prasin
Violaxanthin	Violax
Diadinoxanthin	Diadino
Myoxanthin	Myo
Diatoxanthin	Diato
Echinenone	Ech
Carotene	Car

Table A.2. Summary of the number of points used (n), y-intercept (k), slope (m), r^2 and p values of the linear regression of apparent pigment specific growth rates against the fraction of unfiltered seawater (D) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in March 07. $n < 8$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment marker abbreviations interpretations.

A)

Size fraction	Pigment	n	k (d^{-1})	m (d^{-1})	r^2	p
	Peri	8	0.52	-0.36	0.51	0.045*
	Fuco	8	1.85	-1.42	0.80	0.003**
< 200 µm	19 hex	6	0.25	-0.49	0.28	0.281
	Allo	8	0.30	-0.74	0.91	<0.001**
	Chl *b*	5	1.85	-1.58	0.73	0.065
	Chl *a*	8	1.69	-1.57	0.81	0.002**
	Peri	8	0.81	-0.72	0.81	0.002**
	Fuco	8	1.59	-0.87	0.98	< 0.001**
< 20 µm	Allo	8	0.08	-0.44	0.67	0.044*
	Chl *b*	6	1.00	-0.37	0.46	0.015*
	Chl *a*	8	1.52	-1.14	0.95	< 0.001**
	Peri	8	0.92	-0.85	0.61	0.023*
	Fuco	8	1.62	-0.94	0.91	< 0.001**
< 5 µm	19 hex	8	0.68	-1.05	0.75	0.005**
	Allo	8	0.01	-0.53	0.52	0.045*
	Chl *b*	6	1.38	-1.02	0.81	0.015*
	Chl *a*	8	1.56	-1.09	0.95	< 0.001**

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.2. (Continued)

B)

Size fraction	Pigment	n	k (d^{-1})	m (d^{-1})	r^2	p
	Fuco	8	1.50	-0.35	0.59	0.026*
	19 hex	8	1.15	-1.00	0.71	0.009**
< 200 µm	Allo	6	0.05	0.16	0.05	0.654
	Chl *b*	5	0.50	-0.25	0.17	0.518
	Chl *a*	8	1.60	-0.85	0.49	0.052
	Fuco	8	1.42	-0.97	0.79	0.003**
	19 hex	8	1.22	-1.05	0.72	0.008**
< 20 µm	Allo	6	0.13	-0.14	0.03	0.694
	Chl *b*	4	0.48	-0.27	0.04	0.809
	Chl *a*	8	1.16	-0.78	0.73	0.007**
	Fuco	8	1.31	-0.43	0.33	0.140
	19 hex	8	1.11	-0.95	0.89	< 0.001**
< 5 µm	Allo	4	0.17	-0.00	0.00	0.995
	Chl *b*	6	0.55	-0.42	0.23	0.334
	Chl *a*	8	1.29	-0.65	0.54	0.039*

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.3. Summary of the number of points used (n), y-intercept (k), slope (m), r^2 and p values of the linear regression of apparent pigment specific growth rates against the fraction of unfiltered seawater (D) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in May 07. $n < 8$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	n	k (d^{-1})	m (d^{-1})	r^2	p
	Peri	8	0.68	-0.43	0.49	0.055
	Fuco	8	2.03	-0.88	0.81	0.002**
< 200 µm	19 hex	8	1.58	-0.87	0.22	0.237
	Allo	8	0.53	-0.15	0.02	0.715
	Chl *b*	5	3.56	-2.74	0.54	0.160
	Chl *a*	8	1.8401	-0.94	0.71	0.009**
	Peri	7	2.52	-1.86	0.73	0.014*
	Fuco	8	1.93	-0.83	0.79	0.003**
< 20 µm	19 hex	8	1.68	-0.75	0.77	0.004**
	Allo	8	0.90	-0.94	0.62	0.020*
	Chl *b*	6	6.67	-6.40	0.48	0.126
	Chl *a*	8	1.79	-0.82	0.78	0.004**
	Fuco	8	1.88	-0.74	0.62	0.021*
	19 hex	8	2.12	-1.41	0.86	0.001**
< 5 µm	Allo	8	0.31	-0.33	0.19	0.274
	Chl *b*	6	2.07	-0.73	0.46	0.141
	Chl *a*	8	1.88	-0.98	0.76	0.005**

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.3. (Continued)

B)

Size fraction	Pigment	*n*	k (d^{-1})	m (d^{-1})	r^2	*p*
	Fuco	8	2.28	-0.14	0.22	0.240
< 200 µm	19 hex	4	0.15	-0.03	0.02	0.852
	Lutein	8	0.08	0.03	0.01	0.862
	Chl *a*	8	2.20	-0.61	0.81	0.002**
	Fuco	8	2.15	-0.48	0.70	0.010**
< 5 µm	19 hex	5	0.98	-0.76	0.34	0.303
	Lutein	6	0.43	-0.18	0.10	0.540
	Chl *a*	8	1.88	-0.67	0.92	< 0.001**
	Fuco	8	2.18	-0.11	0.02	0.744
< 200 µm	19 hex	5	1.03	-0.95	0.62	0.116
	Lutein	7	0.43	-0.22	0.09	0.503
	Chl *a*	8	2.10	-0.52	0.45	0.068

** Significant at the 0.01 level

Table A.4. Summary of the number of points used (n), y-intercept (k), slope (m), r^2 and p values of the linear regression of apparent pigment specific growth rates against the fraction of unfiltered seawater (D) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in August 07. $n < 8$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	n	k (d^{-1})	m (d^{-1})	r^2	p
	Peri	8	1.75	-1.18	0.58	0.028*
	Fuco	8	2.86	-1.56	0.57	0.030*
< 200 µm	Allo	7	1.15	-0.82	0.32	0.183
	Chl *b*	6	2.36	-1.86	0.31	0.255
	Chl *a*	8	2.61	-1.87	0.57	0.218
	Peri	8	2.08	-2.92	0.84	0.001**
	Fuco	8	1.90	-1.99	0.96	< 0.001**
< 20 µm	Allo	8	0.11	-1.29	0.69	0.011*
	Zea	4	-1.93	2.77	0.84	0.085
	Chl *b*	4	2.45	-3.73	0.93	0.035*
	Chl *a*	8	2.02	-2.12	0.94	< 0.001**
	Peri	7	0.91	-1.20	0.22	0.294
< 5 µm	Fuco	8	2.27	-1.93	0.77	0.004**
	Allo	8	0.34	-1.08	0.55	0.035*
	Chl *a*	8	2.44	-2.26	0.83	0.002**

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.4. (Continued)

B)

Size fraction	Pigment	*n*	k (d^{-1})	m (d^{-1})	r^2	*p*
	Peri	8	0.95	-0.46	0.31	0.151
	Fuco	8	2.51	-1.38	0.73	0.007**
< 200 µm	Prasin	8	1.93	-1.48	0.79	0.003**
	Allo	6	1.25	-1.12	0.84	0.011*
	Lutein	7	0.45	0.48	0.43	0.110
	Chl *b*	6	0.86	-0.29	0.21	0.355
	Chl *a*	8	2.50	-1.44	0.80	0.003**
	Peri	4	0.36	-0.05	0.11	0.669
	Fuco	8	1.98	-0.87	0.37	0.112
< 20 µm	Prasin	8	1.94	-1.62	0.73	0.007**
	Allo	7	0.45	-0.02	0.00	0.889
	Lutein	7	0.39	0.55	0.61	0.039*
	Chl *b*	4	0.98	-0.66	0.91	0.046*
	Chl *a*	8	2.06	-1.01	0.70	0.009**
	Fuco	8	2.50	-1.64	0.59	0.025*
	Prasin	8	1.78	-1.35	0.78	0.004**
< 5 µm	Allo	6	0.65	-0.45	0.16	0.425
	Lutein	6	0.62	0.24	0.04	0.691
	Chl *a*	8	2.35	-1.49	0.64	0.017*

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.5. Summary of the number of points used (n), y-intercept (k), slope (m), r^2 and p values of the linear regression of apparent pigment specific growth rates against the fraction (D) of unfiltered seawater of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in September 07. $n < 8$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	n	k (d^{-1})	m (d^{-1})	r^2	p
	Peri	8	0.50	-0.15	0.12	0.401
	Fuco	8	1.65	-0.53	0.57	0.030*
< 200 µm	Allo	8	0.59	0.02	0.00	0.952
	Zea	5	2.09	-2.52	0.78	0.048*
	Chl *a*	8	1.66	-1.45	0.80	0.003**
	Peri	8	0.65	-0.59	0.74	0.006**
	Fuco	8	1.80	-1.04	0.86	0.001**
< 20 µm	19 hex	8	1.21	-1.01	0.61	0.022*
	Allo	8	0.32	-0.19	0.06	0.576
	Zea	7	0.62	0.15	0.02	0.754
	Chl *a*	8	1.83	-1.16	0.83	0.002**
	Fuco	8	1.81	-1.26	0.19	0.286
	19 hex	7	0.93	-1.08	0.43	0.112
< 5 µm	Allo	7	0.37	-0.32	0.03	0.700
	Zea	8	1.22	-0.52	0.17	0.313
	Chl *a*	8	1.92	-1.60	0.27	0.185

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.5. (Continued)

B)

Size fraction	Pigment	*n*	k (d^{-1})	m (d^{-1})	r^2	*p*
	Peri	7	0.99	-0.96	0.80	0.007**
	Fuco	8	2.19	-0.56	0.88	0.001**
< 200 µm	19 hex	8	1.25	-0.97	0.80	0.003**
	Allo	8	0.83	-0.70	0.78	0.004**
	Zea	8	1.08	-0.49	0.28	0.181
	Chl *a*	8	1.72	-0.64	0.91	< 0.001**
	Fuco	8	2.05	-0.82	0.72	0.008**
	19 hex	8	1.38	-1.50	0.82	0.002**
< 20 µm	Prasin	5	0.12	0.10	0.01	0.864
	Allo	8	1.09	-1.20	0.73	0.007**
	Zea	8	1.17	-0.45	0.24	0.219
	Chl *a*	8	1.55	-0.75	0.75	0.006**
	Fuco	8	2.36	-1.36	0.84	0.001**
	19 hex	8	1.38	-1.52	0.92	< 0.001**
< 5 µm	Allo	8	1.04	-1.18	0.79	0.003**
	Zea	8	1.30	-0.75	0.40	0.094
	Chl *a*	8	1.86	-1.34	0.92	< 0.001**

** Significant at the 0.01 level

Table A.6. Summary of the number of points used (n), y-intercept (k), slope (m), r^2 and p values of the linear regression of apparent pigment specific growth rates against the fraction (D) of unfiltered seawater of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in November 07. $n < 8$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	n	k (d^{-1})	m (d^{-1})	r^2	p
	Peri	8	-0.48	-0.06	0.04	0.633
	Fuco	8	1.05	-0.63	0.80	0.003**
< 200 µm	19 hex	8	0.52	-0.88	0.91	< 0.001**
	Allo	8	0.38	-0.72	0.81	0.002**
	Zea	5	1.54	-1.66	0.96	0.003**
	Chl *a*	8	0.80	-0.74	0.90	< 0.001**
	Peri	7	-1.01	0.24	0.16	0.367
	Fuco	8	0.80	-0.81	0.81	0.002**
< 20 µm	19 hex	8	0.48	-0.77	0.92	< 0.001**
	Allo	8	0.53	-0.82	0.81	0.002**
	Chl *a*	8	0.46	-0.58	0.65	0.015*
	Peri	8	-0.65	-0.10	0.01	0.843
	Fuco	8	0.74	-0.65	0.19	0.278
< 5 µm	19 hex	8	0.38	-0.49	0.25	0.203
	Allo	8	0.50	-0.75	0.83	0.002**
	Zea	4	-0.02	0.19	0.12	0.654
	Chl *a*	8	0.67	-0.87	0.46	0.064

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.6. (Continued)

B)

Size fraction	Pigment	*n*	k (d^{-1})	m (d^{-1})	r^2	*p*
	Peri	6	0.53	0.08	0.00	0.948
	Fuco	8	3.28	-1.15	0.88	0.001**
< 200 µm	19 hex	7	2.47	-0.96	0.60	0.042*
	Allo	7	1.81	-0.95	0.28	0.220
	Zea	6	2.23	-1.12	0.64	0.056
	Chl *a*	8	2.87	-1.22	0.84	0.001**
	Fuco	8	2.97	-1.12	0.82	0.002**
	19 hex	7	2.28	-0.92	0.63	0.032*
< 20 µm	Allo	7	1.00	-0.30	0.06	0.594
	Zea	5	2.35	-1.03	0.45	0.219
	Chl *a*	8	2.68	-1.25	0.91	< 0.001**
	Fuco	8	2.93	-0.93	0.59	0.025*
	19 hex	6	2.74	-1.38	0.71	0.036*
< 5 µm	Allo	7	1.59	-0.66	0.16	0.381
	Zea	5	1.83	-0.31	0.17	0.487
	Chl *a*	8	2.80	-1.22	0.74	0.006**

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.7. Summary of the number of points used (n), y-intercept (k), slope (m), r^2 and p values of the linear regression of apparent pigment specific growth rates against the fraction of unfiltered seawater (D) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in January 08. $n < 8$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	n	k (d^{-1})	m (d^{-1})	r^2	p
	Peri	8	0.09	0.03	0.01	0.824
	Fuco	8	1.63	-0.58	0.77	0.004**
< 200 µm	Prasin	8	0.53	-0.28	0.57	0.031*
	Allo	8	0.13	-0.19	0.09	0.468
	Chl *b*	6	0.74	-0.52	0.17	0.410
	Chl *a*	8	1.62	-0.79	0.94	< 0.001**
	Peri	8	-0.04	0.22	0.19	0.285
	Fuco	8	0.40	2.29	0.22	0.239
< 20 µm	Prasin	8	0.41	-0.08	0.08	0.501
	Allo	8	-0.50	0.31	0.20	0.272
	Chl *b*	6	0.07	0.21	0.01	0.839
	Chl *a*	8	0.96	-0.04	0.00	0.869
	Peri	8	0.39	-0.18	0.101	0.429
	Fuco	8	1.47	-0.32	0.16	0.329
< 5 µm	Prasin	8	0.56	-0.33	0.29	0.172
	Allo	8	0.00	-0.20	0.15	0.344
	Chl *b*	5	0.01	0.36	0.17	0.487
	Chl *a*	8	1.45	-0.46	0.37	0.111

* Significant at the 0.05 level

** Significant at the 0.01 level

Table A.7. (Continued)

B)

Size fraction	Pigment	*n*	k (d^{-1})	m (d^{-1})	r^2	*p*
	Peri	8	-0.00	0.10	0.11	0.433
< 200 µm	Fuco	8	0.47	-0.23	0.85	0.001**
	Prasin	7	0.22	0.08	0.04	0.652
	Chl *a*	8	0.41	-0.07	0.19	0.275
	Peri	7	0.27	-0.09	0.07	0.577
< 20 µm	Fuco	8	0.34	-0.15	0.30	0.163
	Prasin	6	0.17	0.01	0.00	0.975
	Chl *a*	8	0.15	0.13	0.11	0.418
	Fuco	8	0.21	0.05	0.02	0.762
< 5 µm	Prasin	5	-0.21	0.36	0.36	0.281
	Chl *a*	8	0.28	-0.13	0.07	0.530

** Significant at the 0.01 level

Table A.8. Summary of the revised estimated pigment specific microzooplankton grazing rate (g), phytoplankton potential growth rate (μ_n) and phytoplankton growth rate in ambient nutrients (μ_0), and % of standing stock (SS grazed) and production grazed (Production grazed) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in March 07. Not available (N.A.) data due to negative μ_0. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.36	0.52	0.38	30.3	95.5
	Fuco	1.42	1.85	1.77	75.8	91.3
< 200	19 hex	0.00	-0.11	0.17	0.0	0.0
µm	Allo	0.74	0.30	0.35	52.1	175.8
	Chl *b*	0.00	0.21	0.19	0.0	0.0
	Chl *a*	1.57	1.69	1.67	79.2	97.6
	Peri	0.72	0.81	0.56	51.3	119.6
	Fuco	0.87	1.59	0.89	58.1	98.2
< 20 µm	Allo	0.44	0.08	-0.08	35.7	N.A.
	Chl *b*	0.37	1.00	0.35\	30.7	103.1
	Chl *a*	1.14	1.52	0.94\	68.0	111.2
	Peri	0.85	0.92	0.55	57.3	135.1
	Fuco	0.94	1.62	1.04	60.9	94.4
< 5 µm	19 hex	1.05	0.68	1.28	65.0	90.1
	Allo	0.53	0.01	0.01	41.4	3573.7
	Chl *b*	1.02	1.38	0.92	63.9	105.8
	Chl *a*	1.09	1.56	1.04	66.3	102.7

Table A.8. (Continued)

B)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Fuco	0.35	1.50	0.69	29.7	59.7
	19 hex	1.00	1.15	1.08	63.1	95.6
< 200	Allo	0.00	0.11	-0.05	0.0	0.0
µm	Chl *b*	0.00	0.25	-0.22	0.0	0.0
	Chl *a*	0.00	0.76	0.19	0.0	0.0
	Fuco	0.97	1.42	1.03	62.1	96.9
	19 hex	1.05	1.22	1.06	64.9	99.6
< 20 µm	Allo	0.00	0.14	-0.19	0.0	0.0
	Chl *b*	0.00	0.21	0.00	0.0	0.0
	Chl *a*	0.78	1.16	0.78	54.2	100.2
	Fuco	0.00	0.90	0.08	0.0	0.0
	19 hex	0.95	1.11	0.99	61.3	97.2
< 5 µm	Allo	0.00	0.16	0.11	0.0	0.0
	Chl *b*	0.00	0.10	-0.23	0.0	0.0
	Chl *a*	0.65	1.29	0.58	47.9	108.4

Table A.9. Summary of the revised estimated pigment specific microzooplankton grazing rate (g), phytoplankton potential growth rate (μ_n) and phytoplankton growth rate in ambient nutrients (μ_0), and % of standing stock (SS grazed) and production grazed (Production grazed) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in May 07. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.00	0.37	-0.93	0.0	0.0
	Fuco	0.88	2.03	0.71	58.4	115.2
< 200	19 hex	0.00	0.80	0.14	0.0	0.0
µm	Allo	0.00	0.44	-0.41	0.0	0.0
	Chl *b*	0.00	0.72	-0.59	0.0	0.0
	Chl *a*	0.94	1.84	0.71	60.8	120.1
	Peri	1.86	2.52	1.75	84.5	102.3
	Fuco	0.83	1.93	0.32	56.4	205.3
< 20 µm	19 hex	0.75	1.68	0.69	53.0	106.8
	Allo	0.94	0.90	0.08	60.8	797.4
	Chl *b*	0.00	0.58	-1.08	0.0	0.0
	Chl *a*	0.82	1.79	0.19	56.0	319.2
	Fuco	0.74	1.88	0.46	52.4	140.9
	19 hex	1.41	2.12	1.40	75.6	100.2
< 5 µm	Allo	0.00	0.02	-0.52	0.0	0.0
	Chl *b*	0.00	1.36	-0.44	0.0	0.0
	Chl *a*	0.98	1.88	0.62	62.6	135.1

Table A.9. (Continued)

A)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
< 200 µm	Fuco	0.00	2.17	1.25	0.0	0.0
	19 hex	0.00	0.07	0.23	0.0	0.0
	Lutein	0.00	0.05	-0.47	0.0	0.0
	Chl *a*	0.61	2.20	1.48	45.5	58.9
< 20 µm	Fuco	0.48	2.15	1.48	38.4	49.7
	19 hex	0.00	0.13	-0.01	0.0	0.0
	Lutein	0.00	0.28	-0.24	0.0	0.0
	Chl *a*	0.67	1.88	1.25	48.6	68.2
< 5 µm	Fuco	0.00	2.06	1.38	0.0	0.0
	19 hex	0.00	-0.02	0.19	0.0	0.0
	Lutein	0.00	0.20	-0.44	0.0	0.0
	Chl *a*	0.00	1.65	1.02	0.0	0.0

Table A.10. Summary of the revised estimated pigment specific microzooplankton grazing rate (g), phytoplankton potential growth rate (μ_n) and phytoplankton growth rate in ambient nutrients (μ_0), and % of standing stock (SS grazed) and production grazed (Production grazed) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in August 07. Not available (N.A.) data due to negative μ_0. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	1.18	1.75	0.62	69.2	149.0
	Fuco	1.56	2.86	1.45	79.0	103.3
< 200	Allo	0.00	0.33	-0.53	0.0	0.0
µm	Chl *b*	0.00	0.29	-1.29	0.0	0.0
	Chl *a*	0.00	0.67	-0.34	0.0	0.0
	Peri	2.92	2.08	1.24	94.6	133.0
	Fuco	1.99	1.90	1.28	86.3	119.4
< 20 µm	Allo	1.29	0.11	-0.42	72.5	N.A.
	Zea	0.00	0.83	0.16	0.0	0.0
	Chl *b*	3.73	2.45	3.73	97.6	100.0
	Chl *a*	2.12	2.02	1.28	88.1	121.8
	Peri	0.00	-0.65	-0.14	0.0	0.0
< 5 µm	Fuco	1.93	2.27	1.70	85.5	104.5
	Allo	1.08	0.34	0.35	66.1	225.3
	Chl *a*	2.26	2.44	1.91	89.6	105.1

Table A.10. (Continued)

B)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.00	0.37	0.37	0.0	0.0
	Fuco	1.38	2.51	1.47	74.7	97.1
	Prasin	1.48	1.92	1.77	77.2	93.2
< 200	Allo	1.12	1.25	1.12	67.4	100.3
µm	Lutein	0.00	0.95	0.01	0.0	0.0
	Chl *b*	0.00	0.56	0.22	0.0	0.0
	Chl *a*	1.44	2.50	1.68	76.4	94.0
	Peri	0.00	0.29	0.01	0.0	0.0
	Fuco	0.00	1.021	0.03	0.0	0.0
	Prasin	1.62	1.94	1.99	80.2	92.8
< 20 µm	Allo	0.00	0.42	0.14	0.0	0.0
	Lutein	0.00	0.39	-0.45	0.0	0.0
	Chl *b*	0.66	0.98	0.88	48.3	82.4
	Chl *a*	1.01	2.06	1.16	63.7	92.9
	Fuco	1.64	2.50	1.96	80.6	93.9
	Prasin	1.35	1.78	1.60	74.0	92.7
< 5 µm	Allo	0.00	0.32	0.00	0.0	0.0
	Lutein	0.00	0.96	0.15	0.0	0.0
	Chl *a*	1.49	2.35	1.74	77.4	93.9

Table A.11. Summary of the revised estimated pigment specific microzooplankton grazing rate (g), phytoplankton potential growth rate (μ_n) and phytoplankton growth rate in ambient nutrients (μ_0), and % of standing stock (SS grazed) and production grazed (Production grazed) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in September 07. Not available (N.A.) data due to negative μ_0. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.00	0.29	-0.60	0.0	0.0
	Fuco	0.53	1.65	0.01	41.3	3508.6
< 200	Allo	0.00	0.52	-0.38	0.0	0.0
µm	Zea	2.52	2.09	2.11	92.0	104.7
	Chl *a*	1.45	1.66	0.30	76.5	298.1
	Peri	0.59	0.65	-0.08	44.8	N.A.
	Fuco	1.04	1.80	0.37	64.8	210.3
	19 hex	1.01	1.21	0.98	63.5	101.5
< 20 µm	Allo	0.00	0.31	-0.57	0.0	0.0
	Zea	0.00	0.64	-0.13	0.0	0.0
	Chl *a*	1.16	1.83	0.40	68.6	210.3
	Fuco	0.00	0.63	-0.76	0.0	0.0
	19 hex	0.00	-0.02	-0.21	0.0	0.0
< 5 µm	Allo	0.00	0.00	-1.00	0.0	0.0
	Zea	0.00	0.71	0.01	0.0	0.0
	Chl *a*	0.00	0.40	-0.84	0.0	0.0

Table A.11. (Continued)

B)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.96	0.99	1.09	61.6	92.7
	Fuco	0.56	2.19	0.93	43.2	71.3
< 200	19 hex	0.97	1.25	0.95	62.0	101.3
µm	Allo	0.70	0.83	0.20	50.5	283.0
	Zea	0.00	0.74	0.12	0.0	0.0
	Chl *a*	0.64	1.72	0.66	47.3	98.1
	Fuco	0.82	2.05	0.86	55.9	96.8
	19 hex	1.50	1.38	1.28	77.7	107.5
< 20 µm	Allo	1.20	1.09	0.54	69.9	167.3
	Zea	0.00	0.84	-0.21	0.0	0.0
	Chl *a*	0.75	1.55	0.42	52.9	155.4
	Fuco	1.36	2.36	1.40	74.3	98.7
	19 hex	1.52	1.38	1.25	78.2	109.4
< 5 µm	Allo	1.18	1.04	0.62	69.3	149.9
	Zea	0.00	0.77	-0.11	0.0	0.0
	Chl *a*	1.34	1.86	1.02	73.8	115.7

Table A.12. Summary of the revised estimated pigment specific microzooplankton grazing rate (g), phytoplankton potential growth rate (μ_n) and phytoplankton growth rate in ambient nutrients (μ_0), and % of standing stock (SS grazed) and production grazed (Production grazed) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in November 07. Not available (N.A.) data due to negative μ_0. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.00	-0.58	-0.47	0.0	0.0
	Fuco	0.63	1.05	0.44	46.9	132.8
< 200	19 hex	0.88	0.52	0.48	58.5	154.0
µm	Allo	0.72	0.38	0.11	51.2	503.1
	Zea	1.66	1.54	1.38	81.0	108.1
	Chl *a*	0.74	0.80	0.31	52.3	198.0
	Peri	0.00	-0.85	-1.18	0.0	0.0
	Fuco	0.81	0.80	0.28	55.6	226.9
< 20 µm	19 hex	0.77	0.48	0.26	53.6	234.8
	Allo	0.82	0.53	0.22	56.0	287.89
	Chl *a*	0.58	0.46	-0.08	43.8	N.A.
	Peri	0.00	-0.63	-0.98	0.0	0.0
	Fuco	0.00	0.13	-0.72	0.0	0.0
< 5 µm	19 hex	0.00	-0.09	-0.44	0.0	0.0
	Allo	0.75	0.50	0.02	52.9	3157.8
	Zea	0.00	0.20	-0.17	0.0	0.0
	Chl *a*	0.00	-0.17	-0.81	0.0	0.0

Table A.12. (Continued)

B)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.00	0.64	0.71	0.0	0.0
	Fuco	1.15	3.28	3.23	68.5	71.3
< 200	19 hex	0.96	2.47	2.46	61.7	67.5
µm	Allo	0.00	0.81	0.66	0.0	0.0
	Zea	0.00	1.12	1.21	0.0	0.0
	Chl *a*	1.22	2.87	2.85	70.4	74.7
	Fuco	1.12	2.97	2.82	67.5	71.7
	19 hex	0.92	2.28	2.07	60.3	68.9
< 20 µm	Allo	0.00	0.80	0.32	0.0	0.0
	Zea	0.00	1.22	1.00	0.0	0.0
	Chl *a*	1.25	2.68	2.51	71.4	77.7
	Fuco	0.93	2.93	2.44	60.6	66.4
	19 hex	1.38	2.74	2.49	74.8	81.6
< 5 µm	Allo	0.00	0.73	0.53	0.0	0.0
	Zea	0.00	1.46	1.18	0.0	0.0
	Chl *a*	1.22	2.80	2.37	70.6	77.8

Table A.13. Summary of the revised estimated pigment specific microzooplankton grazing rate (g), phytoplankton potential growth rate (μ_n) and phytoplankton growth rate in ambient nutrients (μ_0), and % of standing stock (SS grazed) and production grazed (Production grazed) of various size fractions and pigments for the dilution experiments in TH (A) and MB (B) in January 08. Refer to table A.1A for pigment markers abbreviations interpretations.

A)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS grazed (%)	Production grazed (%)
	Peri	0.00	0.06	-0.11	0.0	0.0
	Fuco	0.58	1.63	0.62	43.9	94.6
< 200	Prasin	0.28	0.53	0.32	24.5	90.4
µm	Allo	0.00	-0.04	-0.37	0.0	0.0
	Chl *b*	0.00	0.21	-0.31	0.0	0.0
	Chl *a*	0.79	1.62	0.78	54.4	100.8
	Peri	0.00	0.21	-0.23	0.0	0.0
	Fuco	0.00	2.52	1.45	0.0	0.0
< 20 µm	Prasin	0.00	0.33	0.02	0.0	0.0
	Allo	0.00	-0.29	-0.46	0.0	0.0
	Chl *b*	0.00	0.24	-0.04	0.0	0.0
	Chl *a*	0.00	0.91	-0.07	0.0	0.0
	Peri	0.00	0.25	-0.30	0.0	0.0
	Fuco	0.00	1.22	-0.05	0.0	0.0
< 5 µm	Prasin	0.00	0.26	-0.10	0.0	0.0
	Allo	0.00	-0.21	-0.62	0.0	0.0
	Chl *b*	0.00	0.30	-0.32	0.0	0.0
	Chl *a*	0.00	1.02	-0.13	0.0	0.0

Table A.13. (Continued)

B)

Size fraction	Pigment	g (d^{-1})	μ_n (d^{-1})	μ_0 (d^{-1})	SS removed (%)	Production removed (%)
	Peri	0.00	0.13	0.03	0.0	0.0
< 200	Fuco	0.23	0.47	0.45	20.4	56.6
µm	Prasin	0.00	0.26	0.10	0.0	0.0
	Chl *a*	0.00	0.31	0.25	0.0	0.0
	Peri	0.00	0.17	0.11	0.0	0.0
< 20 µm	Fuco	0.00	0.19	0.21	0.0	0.0
	Prasin	0.00	0.14	0.08	0.0	0.0
	Chl *a*	0.00	0.22	0.21	0.0	0.0
	Fuco	0.00	0.21	0.09	0.0	0.0
< 5 µm	Prasin	0.00	0.10	-0.02	0.0	0.0
	Chl *a*	0.00	0.11	0.03	0.0	0.0

Table A.14. Summary of Pearson's correlation analyses of various on site physio-chemical parameters against the initial pigment concentrations of various size fractions and pigments. TH and MB data are combined due to small sample size (n). $n < 12$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

Parameter	Size fraction	Pigment	n	Pearson Correlation Coefficient	p
Temperature		Peri	12	-0.184	0.567
		Fuco	12	0.084	0.794
		19 hex	12	0.053	0.870
	< 200 µm	Allo	12	-0.176	0.584
		Zea	7	0.847	0.016*
		Chl *b*	7	0.190	0.683
		Chl *a*	12	-0.148	0.646
		Peri	10	-0.442	0.201
		Fuco	12	-0.130	0.687
		19 hex	12	-0.266	0.404
	< 20 µm	Allo	11	-0.421	0.197
		Zea	6	0.545	0.263
		Chl *b*	6	0.160	0.763
		Chl *a*	12	-0.254	0.425
		Peri	10	-0.594	0.070
		Fuco	12	-0.187	0.561
		19 hex	12	-0.230	0.472
	< 5 µm	Allo	12	-0.255	0.424
		Zea	6	0.539	0.270
		Chl *b*	7	-0.500	0.254
		Chl *a*	12	-0.251	0.431

Table A.14. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Salinity		Peri	12	-0.518	0.085
		Fuco	12	-0.643	0.024*
		19 hex	12	-0.361	0.249
	< 200 µm	Allo	12	-0.322	0.308
		Zea	7	-0.319	0.486
		Chl *b*	7	-0.593	0.161
		Chl *a*	12	-0.330	0.295
		Peri	10	-0.360	0.307
		Fuco	12	-0.289	0.362
		19 hex	12	-0.133	0.680
	< 20 µm	Allo	11	-0.211	0.534
		Zea	6	0.237	0.650
		Chl *b*	6	-0.487	0.327
		Chl *a*	12	-0.118	0.714
		Peri	10	-0.186	0.607
		Fuco	12	-0.242	0.450
		19 hex	12	-0.110	0.733
	< 5 µm	Allo	12	-0.184	0.567
		Zea	6	0.224	0.670
		Chl *b*	7	0.078	0.0867
		Chl *a*	12	-0.113	0.727
DO		Peri	12	0.360	0.251
		Fuco	12	0.329	0.296
		19 hex	12	0.043	0.895
	< 200 µm	Allo	12	0.204	0.524
		Zea	7	-0.676	0.096
		Chl *b*	7	0.345	0.448
		Chl *a*	12	0.334	0.289

Table A.14. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
DO		Peri	10	0.603	0.065
		Fuco	12	0.205	0.522
		19 hex	12	0.397	0.201
	< 20 μm	Allo	11	0.339	0.308
		Zea	6	-0.757	0.081
		Chl *b*	6	0.149	0.778
		Chl *a*	12	0.296	0.351
		Peri	10	0.592	0.071
		Fuco	12	0.387	0.214
		19 hex	12	0.404	0.193
	< 5 μm	Allo	12	0.206	0.520
		Zea	6	-0.782	0.066
		Chl *b*	7	0.375	0.408
		Chl *a*	12	0.339	0.281
Secchi depth		Peri	12	-0.484	0.111
		Fuco	12	-0.714	0.009**
		19 hex	12	-0.256	0.422
	< 200 μm	Allo	12	-0.500	0.098
		Zea	7	-0.370	0.414
		Chl *b*	7	-0.652	0.112
		Chl *a*	12	-0.652	0.030*
		Peri	10	-0.598	0.068
		Fuco	12	-0.576	0.050*
		19 hex	12	-0.135	0.676
	< 20 μm	Allo	11	-0.568	0.068
		Zea	6	-0.119	0.823
		Chl *b*	6	-0.819	0.046*
		Chl *a*	12	-0.504	0.095

Table A.14 (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Secchi depth		Peri	10	-0.453	0.189
		Fuco	12	-0.565	0.055
		19 hex	12	-0.102	0.752
	< 5 µm	Allo	12	-0.443	0.149
		Zea	6	-0.128	0.809
		Chl *b*	7	-0.587	0.166
		Chl *a*	12	-0.505	0.094
NH_4^+		Peri	12	0.000	1.000
		Fuco	12	0.223	0.486
		19 hex	12	-0.308	0.331
	< 200 µm	Allo	12	0.064	0.844
		Zea	7	0.480	0.276
		Chl *b*	7	0.894	0.007**
		Chl *a*	12	0.068	0.834
		Peri	10	0.287	0.422
		Fuco	12	0.078	0.809
		19 hex	12	-0.205	0.523
	< 20 µm	Allo	11	0.376	0.254
		Zea	6	0.206	0.696
		Chl *b*	6	0.711	0.113
		Chl *a*	12	0.036	0.911
		Peri	10	0.108	0.766
		Fuco	12	0.075	0.816
		19 hex	12	-0.217	0.497
	< 5 µm	Allo	12	0.031	0.923
		Zea	6	0.177	0.737
		Chl *b*	7	0.526	0.225
		Chl *a*	12	0.032	0.920

Table A.14. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
$NO_2^- + NO_3^-$ cotent		Peri	12	-0.067	0.835
		Fuco	12	0.207	0.518
		19 hex	12	-0.251	0.432
	< 200 µm	Allo	12	0.352	0.262
		Zea	7	-0.446	0.316
		Chl *b*	7	0.117	0.803
		Chl *a*	12	0.335	0.287
		Peri	10	0.294	0.410
		Fuco	12	0.423	0.171
		19 hex	12	-0.146	0.650
	< 20 µm	Allo	11	0.395	0.229
		Zea	6	-0.377	0.461
		Chl *b*	6	0.312	0.548
		Chl *a*	12	0.434	0.158
		Peri	10	0.322	0.364
		Fuco	12	0.343	0.274
		19 hex	12	-0.171	0.596
	< 5 µm	Allo	12	0.395	0.203
		Zea	6	-0.395	0.203
		Chl *b*	7	0.748	0.053
		Chl *a*	12	0.394	0.205
Si		Peri	12	-0.180	0.575
		Fuco	12	0.002	0.994
		19 hex	12	-0.244	0.444
	< 200 µm	Allo	12	0.256	0.422
		Zea	7	0.685	0.089
		Chl *b*	7	0.072	0.879
		Chl *a*	12	-0.105	0.745

Table A.14. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Si		Peri	10	0.184	0.611
		Fuco	12	-0.057	0.860
		19 hex	12	-0.401	0.197
	< 20 µm	Allo	11	0.378	0.252
		Zea	6	0.287	0.581
		Chl *b*	6	-0.068	0.899
		Chl *a*	12	-0.088	0.786
		Peri	10	0.052	0.887
		Fuco	12	-0.241	0.450
		19 hex	12	-0.481	0.113
	< 5 µm	Allo	12	0.173	0.590
		Zea	6	0.258	0.621
		Chl *b*	7	0.221	0.634
		Chl *a*	12	-0.189	0.557
PO_4^{2-}		Peri	12	0.276	0.386
		Fuco	12	0.013	0.967
		19 hex	12	0.165	0.608
	< 200 µm	Allo	12	0.341	0.278
		Zea	7	0.618	0.140
		Chl *b*	7	-0.005	0.992
		Chl *a*	12	0.055	0.865
		Peri	10	0.420	0.226
		Fuco	12	-0.078	0.811
		19 hex	12	0.355	0.258
	< 20 µm	Allo	11	0.281	0.402
		Zea	6	0.495	0.318
		Chl *b*	6	-0.264	0.614
		Chl *a*	12	0.047	0.885

Table A.14. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
PO_4^{2-}		Peri	10	0.442	0.201
		Fuco	12	-0.012	0.970
		19 hex	12	0.305	0.335
	< 5 μm	Allo	12	0.282	0.375
		Zea	6	0.508	0.303
		Chl *b*	7	0.131	0.780
		Chl *a*	12	0.034	0.917

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.15. Summary of Pearson's correlation analyses of various on site physio-chemical parameters against the initial densities of various groups obtained from microscopy counts. TH and MB data are combined due to small sample size (*n*). 'Others' refers to all phytoplankton except dinoflagellates and diatoms.

Parameter	Group	*n*	Pearson Correlation Coefficient	*p*
Temperature	Dinoflagellate	12	0.147	0.647
	Diatom	12	-0.095	0.770
	Microzooplankton	12	-0.037	0.910
	Others	12	-0.279	0.379
	Cryptophyte	12	-0.105	0.745
Salinity	Dinoflagellate	12	-0.692	0.013*
	Diatom	12	-0.133	0.680
	Microzooplankton	12	-0.524	0.080
	Other	12	0.002	0.996
	Cryptophyte	12	-0.336	0.286
Dissolved oxygen content	Dinoflagellate	12	0.217	0.498
	Diatom	12	0.185	0.564
	Microzooplankton	12	0.432	0.161
	Other	12	0.208	0.516
	Cryptophyte	12	0.247	0.438
Secchi depth	Dinoflagellate	12	-0.299	0.346
	Diatom	12	-0.465	0.128
	Microzooplankton	12	-0.582	0.047*
	Other	12	-0.381	0.221
	Cryptophyte	12	-0.510	0.090

* Siginificant at the 0.05 level

Table A.16. Summary of Pearson's correlation analyses of various on site physio-chemical parameters against the estimated pigment specific phytoplankton potential growth rates (μ_n) of various size fractions and pigments. Correlations with ambient nutrients were not analyzed since μ_n were estimated from enriched incubations. TH and MB data are combined due to small sample size (n). $n < 12$ due to low unavailable μ_n data. Refer to table A.1A for pigment markers abbreviations interpretations.

Parameter	Size fraction	Pigment	n	Pearson Correlation Coefficient	p
Temperature		Peri	10	0.477	0.163
		Fuco	12	0.583	0.047*
		19 hex	10	0.488	0.153
	< 200 µm	Allo	10	0.647	0.043*
		Zea	5	0.370	0.540
		Chl *b*	6	0.578	0.230
		Chl *a*	12	0.356	0.256
		Peri	8	0.376	0.358
		Fuco	12	0.254	0.427
		19 hex	10	0.527	0.118
	< 20 µm	Allo	10	0.401	0.251
		Zea	5	-0.003	0.996
		Chl *b*	6	0.638	0.173
		Chl *a*	12	0.602	0.038*

Table A.16. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Temperature		Peri	4	-0.788	0.212
		Fuco	12	0.507	0.093
		19 hex	11	0.282	0.401
	< 5 µm	Allo	10	0.356	0.313
		Zea	4	-0.120	0.880
		Chl *b*	4	0.420	0.580
		Chl *a*	12	0.441	0.151
Salinity		Peri	10	-0.304	0.393
		Fuco	12	-0.146	0.650
		19 hex	10	-0.016	0.965
	< 200 µm	Allo	10	-0.002	0.996
		Zea	5	-0.913	0.030*
		Chl *b*	6	-0.169	0.750
		Chl *a*	12	0.249	0.434
		Peri	8	-0.355	0.388
		Fuco	12	0.110	0.733
		19 hex	10	-0.150	0.680
	< 20 µm	Allo	10	0.182	0.615
		Zea	5	0.043	0.945
		Chl *b*	6	-0.889	0.018
		Chl *a*	12	-0.087	0.788
		Peri	4	0.829	0.171
		Fuco	12	0.050	0.876
		19 hex	11	0.123	0.719
	< 5 µm	Allo	10	0.171	0.637
		Zea	4	0.755	0.245
		Chl *b*	4	-0.688	0.312
		Chl *a*	12	0.017	0.958

Table A.16. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
DO		Peri	10	-0.100	0.783
		Fuco	12	-0.267	0.402
		19 hex	10	-0.488	0.153
	< 200 µm	Allo	10	-0.749	0.013*
		Zea	5	-0.459	0.437
		Chl *b*	6	-0.606	0.202
		Chl *a*	12	-0.334	0.288
		Peri	8	0.079	0.853
		Fuco	12	0.024	0.940
		19 hex	10	-0.486	0.155
	< 20 µm	Allo	10	-0.661	0.037*
		Zea	5	0.007	0.991
		Chl *b*	6	-0.289	0.578
		Chl *a*	12	-0.401	0.197
		Peri	4	0.488	0.512
		Fuco	12	-0.217	0.498
		19 hex	11	-0.189	0.579
	< 5 µm	Allo	10	-0.587	0.075
		Zea	4	0.219	0.781
		Chl *b*	4	-0.499	0.501
		Chl *a*	12	-0.109	0.737
Secchi depth		Peri	10	-0.211	0.559
		Fuco	12	0.085	0.793
		19 hex	10	0.451	0.190
	< 200 µm	Allo	10	0.299	0.402
		Zea	5	-0.746	0.147
		Chl *b*	6	0.026	0.960
		Chl *a*	12	0.201	0.530

Table A.16. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Secchi depth		Peri	8	-0.469	0.241
		Fuco	12	-0.109	0.736
		19 hex	10	0.215	0.551
	< 20 μm	Allo	10	0.015	0.968
		Zea	5	-0.352	0.562
		Chl *b*	6	-0.416	0.412
		Chl *a*	12	0.077	0.812
		Peri	4	-0.324	0.676
		Fuco	12	0.130	0.687
		19 hex	11	0.201	0.553
	< 5 μm	Allo	10	0.201	0.577
		Zea	4	0.094	0.906
		Chl *b*	4	-0.793	0.207
		Chl *a*	12	0.167	0.603

* Siginificant at the 0.05 level

Table A.17. Summary of Pearson's correlation analyses of various on site physio-chemical parameters against the estimated pigment specific phytoplankton growth rates in ambient nutrients (μ_0) of various size fractions and pigments. TH and MB data are combined due to small sample size (n). $n < 12$ due to low unavailable μ_0 data. Refer to table A.1A for pigment markers abbreviations interpretations.

Parameter	Size fraction	Pigment	n	Pearson Correlation Coefficient	p
Temperature		Peri	12	0.138	0.669
		Fuco	12	0.014	0.967
		19 hex	10	0.387	0.269
	< 200 µm	Allo	11	0.176	0.605
		Zea	6	-0.203	0.700
		Chl *b*	6	-0.383	0.454
		Chl *a*	12	-0.076	0.813
		Peri	10	0.223	0.535
		Fuco	12	-0.141	0.662
		19 hex	10	0.519	0.124
	< 20 µm	Allo	10	0.083	0.820
		Zea	5	-0.704	0.185
		Chl *b*	6	0.540	0.268
		Chl *a*	12	0.156	0.628
		Peri	7	0.000	1.000
		Fuco	12	0.323	0.305
		19 hex	11	0.205	0.545
	< 5 µm	Allo	10	0.042	0.909
		Zea	6	-0.269	0.607
		Chl *b*	5	-0.253	0.681
		Chl *a*	12	0.267	0.402

Table A.17. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Salinity		Peri	12	0.186	0.563
		Fuco	12	0.180	0.576
		19 hex	10	0.099	0.785
	< 200 µm	Allo	11	0.294	0.380
		Zea	6	0.079	0.882
		Chl *b*	6	0.687	0.132
		Chl *a*	12	0.447	0.145
		Peri	10	-0.276	0.440
		Fuco	12	0.313	0.322
		19 hex	10	-0.054	0.883
	< 20 µm	Allo	10	0.417	0.231
		Zea	5	0.359	0.553
		Chl *b*	6	-0.763	0.078
		Chl *a*	12	0.175	0.587
		Peri	7	0.297	0.518
		Fuco	12	0.123	0.704
		19 hex	11	0.269	0.424
	< 5 µm	Allo	10	0.245	0.494
		Zea	6	0.188	0.722
		Chl *b*	5	-0.011	0.985
		Chl *a*	12	0.091	0.779
DO		Peri	12	-0.139	0.667
		Fuco	12	0.006	0.985
		19 hex	10	-0.477	0.163
	< 200 µm	Allo	11	-0.406	0.216
		Zea	6	-0.491	0.322
		Chl *b*	6	-0.220	0.676
		Chl *a*	12	-0.094	0.771

Table A.17. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
DO		Peri	10	0.185	0.609
		Fuco	12	0.241	0.451
		19 hex	10	-0.567	0.087
	< 20 µm	Allo	10	-0.420	0.227
		Zea	5	0.561	0.325
		Chl *b*	6	-0.156	0.768
		Chl *a*	12	-0.155	0.631
		Peri	7	0.096	0.838
		Fuco	12	-0.140	0.664
		19 hex	11	-0.186	0.584
	< 5 µm	Allo	10	-0.145	0.690
		Zea	6	0.464	0.354
		Chl *b*	5	-0.167	0.789
		Chl *a*	12	-0.048	0.883
Secchi depth		Peri	12	0.224	0.484
		Fuco	12	0.086	0.791
		19 hex	10	0.518	0.125
	< 200 µm	Allo	11	0.578	0.063
		Zea	6	-0.094	0.860
		Chl *b*	6	0.482	0.334
		Chl *a*	12	0.201	0.531
		Peri	10	-0.468	0.172
		Fuco	12	0.011	0.972
		19 hex	10	0.395	0.259
	< 20 µm	Allo	10	0.264	0.460
		Zea	5	0.253	0.681
		Chl *b*	6	-0.171	0.746
		Chl *a*	12	0.223	0.487

Table A.17. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Secchi depth		Peri	7	0.227	0.624
		Fuco	12	0.177	0.581
		19 hex	11	0.227	0.503
	< 5 µm	Allo	10	0.280	0.433
		Zea	6	-0.020	0.970
		Chl *b*	5	-0.206	0.739
		Chl *a*	12	0.196	0.542
NH_4^+		Peri	12	0.117	0.717
		Fuco	12	-0.152	0.638
		19 hex	10	-0.611	0.061
	< 200 µm	Allo	11	-0.536	0.089
		Zea	6	-0.813	0.049*
		Chl *b*	6	-0.770	0.074
		Chl *a*	12	-0.373	0.232
		Peri	10	0.442	0.200
		Fuco	12	-0.121	0.708
		19 hex	10	-0.552	0.098
	< 20 µm	Allo	10	-0.219	0.543
		Zea	5	-0.346	0.568
		Chl *b*	6	0.563	0.244
		Chl *a*	12	-0.203	0.527
		Peri	7	0.212	0.649
		Fuco	12	0.035	0.914
		19 hex	11	-0.256	0.447
	< 5 µm	Allo	10	0.149	0.681
		Zea	6	0.007	0.989
		Chl *b*	5	0.127	0.839
		Chl *a*	12	0.033	0.918

Table A.17. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
$NO_2^- + NO_3^-$		Peri	12	0.115	0.723
		Fuco	12	0.777	0.003**
		19 hex	10	0.284	0.427
	< 200 µm	Allo	11	0.334	0.316
		Zea	6	0.273	0.600
		Chl *b*	6	0.347	0.501
		Chl *a*	12	0.771	0.003**
		Peri	10	0.110	0.763
	< 20 µm	Fuco	12	0.576	0.050*
		19 hex	10	0.350	0.322
		Allo	10	0.071	0.846
		Zea	5	0.883	0.047*
		Chl *b*	6	-0.155	0.769
		Chl *a*	12	0.669	0.017*
		Peri	7	0.682	0.091
	< 5 µm	Fuco	12	0.461	0.131
		19 hex	11	0.544	0.084
		Allo	10	0.107	0.769
		Zea	6	0.920	0.009**
		Chl *b*	5	0.819	0.090
		Chl *a*	12	0.478	0.116
Si		Peri	12	0.518	0.085
		Fuco	12	0.195	0.544
		19 hex	10	-0.104	0.775
	< 200 µm	Allo	11	-0.138	0.686
		Zea	6	-0.370	0.470
		Chl *b*	6	-0.182	0.730
		Chl *a*	12	-0.034	0.917

Table A.17. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Si		Peri	10	-0.098	0.787
		Fuco	12	0.247	0.440
		19 hex	10	-0.158	0.664
	< 20 µm	Allo	10	-0.164	0.652
		Zea	5	-0.191	0.758
		Chl *b*	6	0.711	0.113
		Chl *a*	12	0.254	0.426
		Peri	7	0.357	0.432
		Fuco	12	0.187	0.560
		19 hex	11	-0.163	0.631
	< 5 µm	Allo	10	0.363	0.302
		Zea	6	0.300	0.564
		Chl *b*	5	0.864	0.059
		Chl *a*	12	0.139	0.667
PO_4^{2-}		Peri	12	0.321	0.310
		Fuco	12	-0.049	0.879
		19 hex	10	-0.347	0.326
	< 200 µm	Allo	11	-0.118	0.731
		Zea	6	-0.563	0.245
		Chl *b*	6	-0.012	0.982
		Chl *a*	12	-0.071	0.826
		Peri	10	-0.351	0.319
		Fuco	12	0.292	0.357
		19 hex	10	-0.500	0.141
	< 20 µm	Allo	10	-0.085	0.816
		Zea	5	-0.549	0.337
		Chl *b*	6	0.264	0.613
		Chl *a*	12	-0.151	0.639

Table A.17. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
PO_4^{2-}		Peri	7	0.003	0.994
		Fuco	12	-0.033	0.918
		19 hex	11	-0.401	0.222
	< 5 μm	Allo	10	0.209	0.562
		Zea	6	-0.578	0.230
		Chl *b*	5	0.315	0.606
		Chl *a*	12	-0.118	0.714

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.18. Summary of Pearson's correlation analyses of various on site physio-chemical parameters against the estimated pigment specific microzooplankton grazing rates (g) of various size fractions and pigments. Correlations with ambient nutrients were not analyzed since g were estimated from enriched incubations. TH and MB data are combined due to small sample size (n). $n < 12$ due to low unavailable g data. Refer to table A.1A for pigment markers abbreviations interpretations.

Parameter	Size fraction	Pigment	n	Pearson Correlation Coefficient	p
Temperature		Peri	10	0.432	0.212
		Fuco	12	0.341	0.278
	< 200 μm	19 hex	10	0.541	0.106
		Allo	10	0.197	0.585
		Chl *a*	12	0.247	0.438
		Peri	8	0.451	0.262
		Fuco	12	0.358	0.254
	< 20 μm	19 hex	10	0.712	0.021*
		Allo	10	0.371	0.292
		Chl *b*	6	0.608	0.200
		Chl *a*	12	0.590	0.043*
		Peri	4	-0.553	0.447
		Fuco	12	0.608	0.036*
	< 5 μm	19 hex	11	0.354	0.286
		Allo	10	0.297	0.405
		Chl *b*	4	-0.494	0.506
		Chl *a*	12	0.513	0.088

Table A.18. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Salinity		Peri	10	-0.364	0.301
		Fuco	12	-0.586	0.045*
	< 200 µm	19 hex	10	-0.156	0.668
		Allo	10	0.022	0.951
		Chl *a*	12	-0.086	0.790
		Peri	8	-0.687	0.060
		Fuco	12	-0.459	0.134
	< 20 µm	19 hex	10	-0.275	0.443
		Allo	10	-0.450	0.192
		Chl *b*	6	-0.866	0.026*
		Chl *a*	12	-0.541	0.070
		Peri	4	0.546	0.454
		Fuco	12	-0.436	0.156
	< 5 µm	19 hex	11	0.123	0.719
		Allo	10	-0.355	0.314
		Chl *b*	4	-0.097	0.903
		Chl *a*	12	-0.377	0.227
DO		Peri	10	-0.142	0.696
		Fuco	12	-0.057	0.860
	< 200 µm	19 hex	10	-0.624	0.054
		Allo	10	-0.403	0.248
		Chl *a*	12	-0.381	0.221
		Peri	8	0.025	0.952
		Fuco	12	-0.221	0.490
	< 20 µm	19 hex	10	-0.766	0.010**
		Allo	10	-0.133	0.714
		Chl *b*	6	-0.154	0.772
		Chl *a*	12	-0.361	0.249

Table A.18. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
DO		Peri	4	-0.032	0.968
		Fuco	12	-0.291	0.358
	< 5 µm	19 hex	11	-0.310	0.354
		Allo	10	-0.213	0.555
		Chl *b*	4	-0.195	0.805
		Chl *a*	12	-0.196	0.542
Secchi depth		Peri	10	-0.224	0.533
		Fuco	12	-0.210	0.512
	< 200 µm	19 hex	10	0.716	0.020*
		Allo	10	0.309	0.385
		Chl *a*	12	-0.091	0.778
		Peri	8	-0.513	0.193
		Fuco	12	-0.277	0.384
	< 20 µm	19 hex	10	0.421	0.226
		Allo	10	-0.457	0.184
		Chl *b*	6	-0.336	0.516
		Chl *a*	12	-0.126	0.696
		Peri	4	-0.466	0.534
		Fuco	12	-0.061	0.851
	< 5 µm	19 hex	11	0.225	0.507
		Allo	10	-0.301	0.397
		Chl *b*	4	-0.413	0.587
		Chl *a*	12	-0.009	0.979

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.19. Summary of Pearson's correlation analyses of various on site physio-chemical parameters against the ratios of the estimated pigment specific microzooplankton grazing rates to the phytoplankton growth rates in ambient nutrients (g/μ_0) of various size fractions and pigments. TH and MB data are combined due to small sample size (n). $n < 12$ due to low unavailable μ_0 data. Refer to table A.1A for pigment markers abbreviations interpretations.

Parameter	Size fraction	Pigment	n	Pearson Correlation Coefficient	p
Temperature		Peri	10	0.300	0.399
		Fuco	12	0.323	0.306
		19 hex	10	0.261	0.466
	< 200 μm	Allo	10	-0.053	0.885
		Zea	5	0.243	0.693
		Chl *a*	12	0.332	0.292
		Peri	7	0.383	0.397
		Fuco	12	0.315	0.319
		19 hex	10	0.262	0.465
	< 20 μm	Allo	8	0.078	0.854
		Chl *b*	6	0.362	0.481
		Chl *a*	11	0.506	0.112
		Peri	4	0.468	0.125
		Fuco	12	0.468	0.125
		19 hex	11	0.308	0.357
	< 5 μm	Allo	10	-0.448	0.194
		Chl *b*	4	-0.494	0.506
		Chl *a*	12	0.333	0.290

Table A.19. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Salinity		Peri	10	-0.470	0.171
		Fuco	12	-0.320	0.310
	< 200 µm	19 hex	10	-0.411	0.238
		Allo	10	-0.053	0.884
		Zea	5	-0.959	0.010**
		Chl *a*	12	-0.385	0.217
		Peri	7	-0.651	0.113
		Fuco	12	-0.545	0.067
	< 20 µm	19 hex	10	-0.547	0.067.
		Allo	8	-0.271	0.516
		Chl *b*	6	-0.555	0.253
		Chl *a*	11	-0.435	0.181
		Peri	4	0.546	0.454
		Fuco	12	-0.375	0.230
	< 5 µm	19 hex	11	0.171	0.614
		Allo	10	-0.093	0.798
		Chl *b*	4	-0.097	0.903
		Chl *a*	12	-0.110	0.733
DO		Peri	10	0.028	0.940
		Fuco	12	-0.469	0.124
	< 200 µm	19 hex	10	-0.307	0.387
		Allo	10	-0.308	0.386
		Zea	5	-0.347	0.567
		Chl *a*	12	-0.518	0.084

Table A.19. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
DO		Peri	7	0.022	0.963
		Fuco	12	-0.386	0.215
	< 20 μm	19 hex	10	-0.447	0.195
		Allo	8	0.066	0.876
		Chl *b*	6	-0.397	0.436
		Chl *a*	11	-0.429	0.188
		Peri	4	-0.032	0.968
		Fuco	12	-0.158	0.624
	< 5 μm	19 hex	11	-0.271	0.420
		Allo	10	0.088	0.808
		Chl *b*	4	-0.195	0.805
		Chl *a*	12	-0.139	0.667
Secchi depth		Peri	10	-0.346	0.328
		Fuco	12	-0.238	0.457
	< 200 μm	19 hex	10	0.387	0.269
		Allo	10	0.024	0.948
		Zea	5	-0.567	0.319
		Chl *a*	12	-0.260	0.415
		Peri	7	-0.571	0.181
		Fuco	12	-0.353	0.260
	< 20 μm	19 hex	10	0.049	0.893
		Allo	8	-0.469	0.241
		Chl *b*	6	-0.262	0.616
		Chl *a*	11	-0.330	0.322

Table A.19. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
Secchi depth		Peri	4	-0.466	0.534
		Fuco	12	-0.290	0.361
	< 5 μm	19 hex	11	0.280	0.404
		Allo	10	-0.229	0.524
		Chl *b*	4	-0.413	0.587
		Chl *a*	12	0.051	0.874
NH_4^+		Peri	10	0.286	0.271
		Fuco	12	-0.283	0.373
		19 hex	10	-0.550	0.100
	< 200 μm	Allo	10	-0.167	0.646
		Zea	5	-0.408	0.495
		Chl *a*	12	-0.450	0.142
		Peri	7	0.283	0.539
		Fuco	12	-0.288	0.364
	< 20 μm	19 hex	10	-0.489	0.151
		Allo	8	0.491	0.216
		Chl *b*	6	0.368	0.473
		Chl *a*	11	-0.190	0.576
		Peri	4	0.000	1.000
		Fuco	12	0.150	0.643
	< 5 μm	19 hex	11	-0.197	0.562
		Allo	10	-0.028	0.940
		Chl *b*	4	0.333	0.667
		Chl *a*	12	-0.006	0.986

Table A.19. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
$NO_2^- + NO_3^-$		Peri	10	-0.044	0.903
		Fuco	12	-0.099	0.760
	< 200 µm	19 hex	10	-0.567	0.087
		Allo	10	-0.302	0.397
		Zea	5	-0.230	0.709
		Chl *a*	12	-0.146	0.651
		Peri	7	0.390	0.387
		Fuco	12	-0.196	0.543
	< 20 µm	19 hex	10	-0.368	0.296
		Allo	8	-0.014	0.973
		Chl *b*	6	0.506	0.306
		Chl *a*	11	-0.123	0.718
		Peri	4	0.977	0.023*
		Fuco	12	0.140	0.665
	< 5 µm	19 hex	11	0.024	0.944
		Allo	10	0.207	0.567
		Chl *b*	4	0.926	0.074
		Chl *a*	12	0.011	0.973
Si		Peri	10	0.513	0.129
		Fuco	12	0.076	0.813
	< 200 µm	19 hex	10	-0.312	0.380
		Allo	10	0.040	0.913
		Zea	5	-0.147	0.814
		Chl *a*	12	-0.164	0.610
		Peri	7	0.292	0.525
		Fuco	12	-0.307	0.332
	< 20 µm	19 hex	10	-0.394	0.260
		Allo	8	-0.700	0.053
		Chl *b*	6	0.765	0.077
		Chl *a*	11	-0.464	0.151

Table A.19. (Continued)

Parameter	Size fraction	Pigment	*n*	Pearson Correlation Coefficient	*p*
		Peri	4	0.557	0.443
		Fuco	12	-0.219	0.494
	< 5 µm	19 hex	11	-0.322	0.334
		Allo	10	0.239	0.506
		Chl *b*	4	0.843	0.157
		Chl *a*	12	-0.277	0.384
PO_4^{2-}		Peri	10	0.395	0.258
		Fuco	12	-0.249	0.436
	< 200 µm	19 hex	10	0.254	0.479
		Allo	10	0.407	0.244
		Zea	5	-0.061	0.922
		Chl *a*	12	-0.159	0.621
		Peri	7	-0.001	0.998
		Fuco	12	-0.261	0.412
	< 20 µm	19 hex	10	-0.073	0.842
		Allo	8	-0.395	0.333
		Chl *b*	6	0.156	0.768
		Chl *a*	11	-0.496	0.120
		Peri	4	-0.086	0.914
		Fuco	12	-0.323	0.306
	< 5 µm	19 hex	11	-0.276	0.411
		Allo	10	0.376	0.284
		Chl *b*	4	0.307	0.693
		Chl *a*	12	-0.294	0.354

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.20. Summary of Pearson's correlation analyses of the initial pigments concentrations of various size fractions and pigments against the estimated pigment specific phytoplankton potential growth rates (μ_n) of the same pigment and size fraction. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable μ_n data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
	Peri	10	-0.477	0.163
	Fuco	12	0.089	0.784
	19 hex	10	-0.209	0.562
< 200 μm	Allo	10	-0.358	0.310
	Zea	5	0.057	0.928
	Chl *b*	6	0.211	0.688
	Chl *a*	12	-0.009	0.978
	Peri	8	-0.302	0.510
	Fuco	12	0.043	0.894
	19 hex	10	-0.312	0.381
< 20 μm	Allo	10	-0.300	0.400
	Zea	5	0.039	0.950
	Chl *b*	6	0.318	0.539
	Chl *a*	12	-0.035	0.915
	Peri	4	0.503	0.497
	Fuco	12	-0.051	0.874
	19 hex	11	-0.215	0.525
< 5 μm	Allo	10	-0.263	0.463
	Zea	4	-0.039	0.961
	Chl *b*	4	0.937	0.063
	Chl *a*	12	-0.026	0.936

Table A.21. Summary of Pearson's correlation analyses of the initial pigment concentrations of various size fractions and pigments against the estimated pigment specific microzooplankton grazing rates (g) of the same pigment and size fraction. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable g data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
< 200 μm	Peri	10	-0.162	0.654
	Fuco	12	0.512	0.089
	19 hex	10	-0.042	0.909
	Allo	10	0.266	0.458
	Chl *a*	12	0.416	0.178
< 20 μm	Peri	7	-0.211	0.650
	Fuco	12	0.225	0.482
	19 hex	10	-0.260	0.469
	Allo	10	0.254	0.479
	Chl *b*	6	0.254	0.627
	Chl *a*	12	0.031	0.925
< 5 μm	Peri	4	0.111	0.889
	Fuco	12	0.015	0.962
	19 hex	11	-0.280	0.404
	Allo	10	0.290	0.416
	Chl *b*	4	0.768	0.232
	Chl *a*	12	0.004	0.991

Table A.22. Summary of Pearson's correlation analyses of the initial pigments concentrations of various size fractions and pigments against the ratios of the estimated pigment specific microzooplankton grazing rates to the phytoplankton growth rates in ambient nutrients (g/μ_0) of the same pigment and size fraction. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable g/μ_0 data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
	Peri	10	-0.031	0.931
	Fuco	12	0.172	0.593
	19 hex	10	0.535	0.111
< 200 μm	Allo	10	0.319	0.368
	Zea	5	0.024	0.970
	Chl *a*	12	0.265	0.404
	Peri	6	-0.111	0.834
	Fuco	12	0.394	0.205
	19 hex	10	0.308	0.387
< 20 μm	Allo	8	0.494	0.214
	Chl *b*	6	-0.023	0.966
	Chl *a*	11	0.351	0.290
	Peri	4	0.111	0.889
	Fuco	12	0.465	0.127
	19 hex	11	-0.251	0.456
< 5 μm	Allo	10	0.821	0.004**
	Chl *b*	4	0.768	0.232
	Chl *a*	12	0.299	0.345

** Significant at the 0.01 level

Table A.23. Summary of Pearson's correlation analyses of the initial densities of microzooplankton against the initial pigment concentrations of various size fractions and pigments. TH and MB data are combined due to small sample size (n). $n < 12$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
	Peri	12	0.586	0.045*
	Fuco	12	0.757	0.004**
	19 hex	12	0.479	0.115
< 200 μm	Allo	12	0.621	0.031*
	Zea	7	0.583	0.170
	Chl *b*	7	0.363	0.423
	Chl *a*	12	0.605	0.037*
	Peri	10	0.813	0.004**
	Fuco	12	0.429	0.164
	19 hex	12	0.385	0.216
< 20 μm	Allo	11	0.574	0.065*
	Zea	6	-0.043	0.936
	Chl *b*	6	0.201	0.703
	Chl *a*	12	0.431	0.162
	Peri	10	0.608	0.062
	Fuco	12	0.482	0.113
	19 hex	12	0.342	0.277
< 5 μm	Allo	12	0.496	0.101
	Zea	6	-0.051	0.924
	Chl *b*	7	0.271	0.557
	Chl *a*	12	0.417	0.178

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.24. Summary of Pearson's correlation analyses of the initial densities of microzooplankton against the groups dinoflagellates, diatoms, others (all phytoplankton except dinoflagellates and diatoms), and cryptophytes. TH and MB data are combined due to small sample size (*n*).

Group	*n*	Pearson's correlation coefficient	*p*
Dinoflagellate	12	0.655	0.021*
Diatom	12	0.152	0.636
Others	12	0.292	0.358
Cryptophyte	12	0.664	0.018*

* Siginificant at the 0.05 level

Table A.25. Summary of Pearson's correlation analyses of the dinoflagellate initial densities against the initial pigment concentrations of various size fractions and pigments. TH and MB data are combined due to small sample size (n). $n < 12$ due to low undetectable pigment concentration by HPLC. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
	Peri	12	0.790	0.002**
	Fuco	12	0.475	0.119
	19 hex	12	0.773	0.003**
< 200 μm	Allo	12	0.253	0.427
	Zea	7	0.167	0.720
	Chl *b*	7	0.281	0.541
	Chl *a*	12	0.287	0.365
	Peri	10	0.729	0.017*
	Fuco	12	0.061	0.852
	19 hex	12	0.677	0.016*
< 20 μm	Allo	11	0.116	0.735
	Zea	6	-0.379	0.459
	Chl *b*	6	0.086	0.871
	Chl *a*	12	0.046	0.888
	Peri	10	0.637	0.048*
	Fuco	12	0.209	0.513
	19 hex	12	0.660	0.019*
< 5 μm	Allo	12	0.119	0.712
	Zea	6	-0.343	0.506
	Chl *b*	7	-0.308	0.501
	Chl *a*	12	0.089	0.783

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.26. Summary of Pearson's correlation analyses of the initial densities of dinoflagellates against the groups diatoms, others (all phytoplankton except dinoflagellates and diatoms), and cryptophytes. TH and MB data are combined due to small sample size (*n*).

Group	*n*	Pearson Correlation Coefficient	*p*
Diatom	12	-0.169	0.599
Others	12	-0.210	0.513
Cryptophyte	12	0.426	0.167

Table A.27. Summary of Pearson's correlation analyses of the microzooplankton initial densities against the estimated pigment specific microzooplankton grazing rates (g) of various size fractions and pigments. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable g data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson's correlation coefficient	p
	Peri	10	0.415	0.233
	Fuco	12	0.302	0.340
< 200 μm	19 hex	10	-0.409	0.240
	Allo	10	-0.231	0.520
	Chl *a*	12	-0.039	0.903
	Peri	8	0.310	0.456
	Fuco	12	0.235	0.462
< 20 μm	19 hex	10	-0.365	0.299
	Allo	10	0.179	0.620
	Chl *b*	6	0.434	0.390
	Chl *a*	12	0.163	0.613
	Peri	4	0.066	0.934
	Fuco	12	0.110	0.734
< 5 μm	19 hex	11	-0.365	0.270
	Allo	10	0.313	0.379
	Chl *b*	4	0.397	0.603
	Chl *a*	12	0.098	0.762

Table A.28. Summary of Pearson's correlation analyses of the dinoflagellate initial densities against the estimated pigment specific microzooplankton grazing rates (g) of various size fractions and pigments. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable g data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
	Peri	10	0.019	0.959
	Fuco	12	0.131	0.685
< 200 μm	19 hex	10	0.044	0.903
	Allo	10	-0.171	0.638
	Chl *a*	12	-0.044	0.892
	Peri	8	0.024	0.955
	Fuco	12	0.110	0.734
< 20 μm	19 hex	10	-0.198	0.584
	Allo	10	0.096	0.792
	Chl *b*	6	0.402	0.430
	Chl *a*	12	0.006	0.985
	Peri	4	-0.968	0.032*
	Fuco	12	-0.111	0.730
< 5 μm	19 hex	11	-0.557	0.075
	Allo	10	0.145	0.690
	Chl *b*	4	-0.359	0.641
	Chl *a*	12	-0.161	0.618

* Siginificant at the 0.05 level

Table A.29. Summary of Pearson's correlation analyses of the estimated pigment specific phytoplankton potential growth rate (μ_n) of various size fractions and pigments against the estimated pigment specific microzooplankton grazing rate (μ_0) of the same pigment and size fraction. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable μ_n and μ_0 data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson's correlation Coefficient	p
	Peri	10	0.627	0.052
	Fuco	12	0.770	0.003**
< 200 μm	19 hex	10	0.950	< 0.001**
	Allo	10	0.748	0.013*
	Zea	5	0.961	0.009**
	Chl *b*	6	-0.010	0.985
	Chl *a*	12	0.859	< 0.001**
	Peri	7	0.976	< 0.001**
	Fuco	12	0.793	0.002**
< 20 μm	19 hex	10	0.932	< 0.001**
	Allo	10	0.813	0.004**
	Zea	5	0.646	0.239
	Chl *b*	6	0.913	0.011*
	Chl *a*	12	0.783	0.003**
	Peri	4	0.781	0.219
	Fuco	12	0.928	< 0.001**
< 5 μm	19 hex	11	0.941	< 0.001**
	Allo	10	0.810	0.005**
	Zea	4	0.903	0.097
	Chl *b*	4	0.466	0.534
	Chl *a*	12	0.939	< 0.001**

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.30. Summary of Pearson's correlation analyses of the estimated pigment specific phytoplankton potential growth rate (μ_n) of various size fractions and pigments against the estimated pigment specific microzooplankton grazing rates (g) of the same pigment and size fraction. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable μ_n and g data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
	Peri	10	0.835	0.003**
	Fuco	12	0.601	0.039*
< 200 μm	19 hex	10	0.785	0.007**
	Allo	10	0.577	0.081
	Chl *a*	12	0.733	0.007**
	Peri	7	0.878	0.004**
	Fuco	12	0.328	0.298
< 20 μm	19 hex	10	0.777	0.008**
	Allo	10	0.405	0.245
	Chl *b*	6	0.961	0.002**
	Chl *a*	12	0.712	0.009**
	Peri	4	0.835	0.165
	Fuco	12	0.749	0.005**
< 5 μm	19 hex	11	0.896	< 0.001**
	Allo	10	0.561	0.091
	Chl *b*	4	0.580	0.420
	Chl *a*	12	0.808	0.001**

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level

Table A.31. Summary of Pearson's correlation analyses of the estimated pigment specific phytoplankton potential growth rate (μ_0) of various size fractions and pigments against the estimated pigment specific microzooplankton grazing rates (g) of the same pigment and size fraction. TH and MB data are combined due to small sample size (n). $n < 12$ due to unavailable μ_0 or g data. Refer to table A.1A for pigment markers abbreviations interpretations.

Size fraction	Pigment	n	Pearson Correlation Coefficient	p
	Peri	10	0.635	0.049*
	Fuco	12	0.551	0.063
< 200 μm	19 hex	10	0.796	0.006**
	Allo	10	0.713	0.021*
	Chl *a*	12	0.633	0.027*
	Peri	8	0.810	0.015*
	Fuco	12	0.319	0.312
< 20 μm	19 hex	10	0.829	0.003**
	Allo	10	0.291	0.414
	Chl *b*	6	0.962	0.002**
	Chl *a*	12	0.589	0.044*
	Peri	4	0.814	0.186
	Fuco	12	0.750	0.005**
< 5 μm	19 hex	11	0.920	< 0.001**
	Allo	10	0.569	0.086
	Chl *b*	4	0.990	0.010**
	Chl *a*	12	0.801	0.002**

* Siginificant at the 0.05 level

** Siginificant at the 0.01 level